

Microbe Selection and Optimizing Process Parameters for Degradation of Glucosinolates in Rapeseed Meal by Box–Behnken Design

Youling Gao^{1*}, Zhengling Qian¹, Lingli Jiang², Caisheng Wang¹ & Guoying Qian¹

¹ College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo 315100, Zhejiang, P. R. China

² Ningbo College of Health Sciences, Ningbo 315100, Zhejiang, P. R. China

*Correspondence: Youling Gao, College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo 315100, Zhejiang, P. R. China. Tel: 86-574-88223277; E-mail: gaoyl@zww.edu.cn

DOI: 10.12735/as.v3i2p01

URL: <http://dx.doi.org/10.12735/as.v3i2p01>

Abstract

The present study applied *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium purpurogenum*, *Trichoderma sp.* MAB-2010b and *Saccharomyces cerevisiae* in solid state fermentation to degrade the glucosinolates in rapeseed meal. In addition, SDS-PAGE was used to determine the effect of hydrolysis of those five microbes on peptide size in rapeseed meal. The results indicated that the solid state fermentation with *S. cerevisiae* degraded the glucosinolates more than those with other microbes. The peptides were hydrolyzed by *S. cerevisiae* to a greater extent than others. Thus the following procedure was just focused on the solid state fermentation with *S. cerevisiae*. Box-Behnken design of response surface methodology was applied to optimize the substrate to water ratio, inoculum amount, and duration. The glucosinolate level in rapeseed meal was as the response. The optimal conditions derived from response surface methodology for *S. cerevisiae* fermentation were: 1.0 of substrate to water ratio, 1.5 mL (equal to 5%) of inoculum amount, and 48 h of duration. The minimum content of glucosinolates was 0.46 $\mu\text{mol/g}$ dry matter. *S. cerevisiae* used in the present study thus exhibit the potential use in large scale solid state fermentation for increasing nutrition quality of rapeseed meal.

Keywords: rapeseed meal, solid state fermentation, *saccharomyces cerevisiae*, box-behnken design, glucosinolates

1. Introduction

Rapeseed meal is the second largest oil-seed meal production after soybean meal in the world, and world production of rapeseed and rapeseed meal were 59.3 and 34.9 million tons annually in 2012/2013 (USDA, 2013). It has a protein content of 38% (Bell, 1984), and is cheaper than the meal from soybean (USDA, 2013). Furthermore, it has lower activity of heat labile antinutrients, e.g. protease inhibitors and lectins (Francis, Makkar, & Becker, 2001). Other antinutrients, mainly glucosinolates, however, limit the nutritional value of rapeseed for fish (Rozaan *et al.*, 1996). Glucosinolates in rapeseed meal have been shown to impair growth and thyroid function in farmed fish species, e.g. rainbow trout (Burel *et al.*, 2000) and red sea bream (Glencross, Hawkins, & Curnow, 2004a; Glencross, Hawkins, & Curnow, 2004b). The inclusion of rapeseed meal in the

diets for farmed fish has been evaluated in some studies (Burel *et al.*, 2001; Glencross *et al.*, 2004a, 2004b; Cheng *et al.*, 2010). The results indicate that glucosinolates and their products in rapeseed meal depressed fish growth and thyroid function (Burel *et al.*, 2001). Reduced growth was mainly caused reduced feed intake due to the bitterness, and progoitrin and sinigrin were considered to be responsible for the bitter taste (Mithen, Dekker, Verkerk, Rabot, & Johnson, 2000). In addition, thiocyanates, thiourea and oxazolidithione reduce iodine availability, thus, inhibit thyroid function (Wallig, Belyea, & Tumbleson, 2002). Nitriles may also negatively influence the health of animals (Tanii, Takayasu, Higashi, Leng, & Saijoh, 2004).

Solid state fermentation (SSF) has been reported to improve the nutritional value of rapeseed meal by breakdown of antinutrients through production of exogenous enzymes, as well as using the antinutrients as substrate for microbial growth (Bau *et al.*, 1994; El-Batal & Karem, 2001; Nair & Duvnjak, 1991; Rozan *et al.*, 1996; Vig & Walia, 2001). SSF also can increase protein content due to the synthesis of single cell protein (Frias, Song, Martinez-Villaluenga, De Mejia, & Vidal-Valverde, 2008; Hong, Lee, & Kim, 2004) and change the essential amino acid profile of the substrate (Yigzaw, Gorton, Solomon, & Akalu, 2004). Besides, the influence of SSF on protein peptide characteristics has been proved. Hong *et al.* (2004) reported that SSF with *Aspergillus oryzae* GB-107 reduced peptide size in soybeans and soybean meals. In order to make the SSF efficient, we used some molds from the different sources. They were *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium purpurogenum*, *Trichoderma sp.* MAB-2010b. In addition, SSF with yeast has been tested for increasing nutritional quality of soybean product (Rashad, Mahmoud, Abdou, & Nooman, 2011). Thus, yeast could be used to increase the nutritional quality of rapeseed meal, and a commercial yeast product (*Saccharomyces cerevisiae*) was evaluated in current experiment.

Response surface methodology is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors, and analyzing optimum conditions of factors for desirable responses. It has been accepted as a convenient method for analyzing optimum conditions due to the minimized cost and time compared with the traditional single variable experiments (Vohra & Satyanarayana, 2002). The Box-Behnken is a design of response surface methodology, and is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design. In this design the treatment combinations are at the midpoints of edges of the process space and at the center. These designs are rotatable (or near rotatable) and require three levels of each factor (Gong *et al.*, 2007).

The aim of the present study thus was to compare the effect of *A. oryzae*, *A. niger*, *P. purpurogenum*, *Trichoderma sp.* MAB-2010b and *S. cerevisiae* on degradation of glucosinolates and change of peptide characteristics in rapeseed meal. The most efficient one was selected for further optimizing SSF conditions in terms of degradation of glucosinolates, including substrate to water ratio, inoculum amount and duration, by employing three-level and three-variable Box-Behnken design.

2. Materials and Methods

2.1. Processing of Rapeseed Meal

Rapeseed meal was purchased from Ningbo Chia Tai Agriculture Co., Ltd (Ningbo, China), and was ground using a FW100 grinder (Tianjin Taisite Instrument Co., Ltd, Tianjin, China) equipped with a 0.4 mm screen. Rapeseed meal was dried carefully (30 min at 60°C) before it was stored in a drier containing silica until use.

2.2. Microbe Preparation

The strains of *A. oryzae* (CICC 40214), *A. niger* (CICC 2089) were purchased from China Centre of Industrial Culture Collection (Beijing, China). *P. purpurogenum* (HQ839781) and *Trichoderma sp.*

MAB-2010b (HQ829121) were isolated by our laboratory. *S. cerevisiae* (product No.: Q/YB.J02.07) was purchased from Angel Yeast Co. Ltd (Yichang, China). *A. oryzae*, *A. niger*, *P. purpurogenum* and *Trichoderma* sp. MAB-2010b were transferred to Potato-Dextrose Agar (PDA) medium (Xinran Biological, Shanghai, China). The strain then was incubated at 28°C for 7 days. The spores on the surface of PDA medium were scratched by an inoculation loop and washed carefully by 1-mL sterile water. The washed spores were further served as the inoculum (CFU was adjusted to be 1×10^7 by adding sterile water) for SSF. *S. cerevisiae* preparation was carried out according to the product specification. In general, the dry powder (5 g) was mixed with distilled water (50 mL) and dextrose (1 g) in a 100 mL beaker, and was incubated at 40°C for 1h. The CFU was adjusted to be 1×10^7 by adding sterile water.

2.3. Solid State Fermentation

Rapeseed meal (30 g) mixed with distilled water (30 mL) was transferred to a 150 mL incubation flask (ZP9-150, Shanghai Jiafeng Co. Ltd, Shanghai, China) equipped with ventilated plug as substrate. The inoculum amount was 1 mL for *A. oryzae*, *A. niger*, *P. purpurogenum*, *Trichoderma* sp. MAB-2010b, and *S. cerevisiae*. The incubation was performed at 28°C in a QYC-211 incubator (Shanghai Fuma Laboratory Equipment Co. LTD, Shanghai, China) for 72 h in triplicate. The fermented rapeseed meal was freeze-dried before it was ground and maintained at 4°C until further chemical analysis.

2.4. Optimization of SSF

The most efficient microbe in degradation of glucosinolates and change of peptide characteristics in rapeseed meal was selected for optimizing SSF conditions. The Box-Behnken design with three independent variables (substrate to water ratio, inoculum amount and duration) of SSF and three levels, including five replicates at the central point, was used for fitting a second-order response surface. The range and levels of the independent variables in the Box-Behnken design are indicated in Table 1. A total of 15 runs were used to optimize the three independent variables. Each run was conducted in triplicate. The glucosinolate content in rapeseed meal after each run was taken as the dependent variable, and the mean value of three observations was used.

Table 1. Level and code of independent variables chosen for Box-Behnken design

Variables	Code	Levels		
		-1	0	1
Substrate to water ratio	A	0.5	1	1.5
Inoculum amount (mL)	B	0.5	1	1.5
Duration (h)	C	48	72	96

A quadratic polynomial regression model was assumed for the dependent variable. The model proposed for each response of Y was:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \quad (1)$$

Where Y is the dependent variable, x_i and x_j are independent variables, represents the intercept term, represents the linear effect, represents the squared effect and shows the interaction effect. The Design Expert 7.1.3 (trial version, Stat Ease Inc., Minneapolis, USA) computer program was used for estimating the coefficients of Equation (1) by regression analysis of the experimental data.

2.5. Determination of Glucosinolates

The determination of glucosinolates was conducted in accordance with the palladium oxide method developed by Wang (2009). In brief, 100 g rapeseed meal was placed in a 100 mL centrifuge tube, and treated with water bath (100°C, 10 min). Afterwards, 8 mL distilled water (90°C) was added to the centrifuge tube, which was then placed in a water bath at 100°C for 30 min. The tube was stirred twice during incubation. After cooling, 2 mL distilled water was added to the centrifuge tube, and mixed with the treated rapeseed meal. The filtrate (0.5 mL) was collected to a 10 mL cuvette with plug. Sodium carboxymethyl cellulose solution (2 mL, 0.1%) and palladium chloride solution (1 mL, 0.004M) was added to the the cuvette in sequence. After being shaken, the filtrate was placed in an incubator (24°C, 3 h). The spectrophotomete was used to measure the absorption of the samples at 540 nm. Sodium carboxymethyl cellulose solution and palladium chloride solution were served as the reference solutions. The content of glucosinolates calculated as: $185.2 \times \text{absorption value} + 0.2$, in which 185.2 was obtained by the standard curve, and 0.2 was the adjust value.

2.6. Electrophoresis

Proteins in rapeseed meal and fermented rapeseed meal were extracted by a modified method based on Aluko and McIntosh (2001). Samples were finely ground with the A11 basic Analytical mill. Ground samples (125 mg) were mixed with 2.5 mL of 50 mM Tris-HCl (pH 8.0) containing 1% SDS, 5 mM DTT and 5 µg/mL protease inhibitor. Samples were then incubated on ice for 3 h, followed by 8 consecutive ultrasonications with ice, each lasting 1 min. Samples were kept on ice during ultrasonication. The samples were thereafter transferred to a 1.5-mL microtube containing glass beads (Qiagen, Hilden, Germany) and further disrupted in a FastPrep machine (FP120, BIO101, Savant) at 6,500 g for 45 sec, 5 time repeats with 1 min interval, on ice. The samples were then treated in an ultrasonication water bath (2 cycles of 5 min) prior to centrifugation at 16,000 g for 30 min at 4°C (Centrifuge 5415 R, Eppendorf, Hamburg, Germany). The supernatants were transferred to Eppendorf tubes and kept at -20°C overnight. The samples were concentrated from 380 to 40 µl using Nanosep® Centrifugal Devices (Pall Life Sciences, Ann Arbor, USA) before mixing with 40 µl SDS-PAGE loading buffer, and incubation at 65 °C for 5 min (Thermomixer comfort, Eppendorf, Hamburg, Germany). Mixed samples were then centrifuged at 16,000 g for 15 min at 4°C.

The polyacrylamide separating gel (12%) and stacking gel were prepared by using P0012A gel parparation kit (Beyotime, Jiansu, China). SDS-PAGE was conducted in a Bio-Rad Electrophoresis system (Bio-Rad, Hercules, CA) with polyacrylamide separating gels in 20x dilution of XT MOPS running buffer. Ten µl of samples were loaded respectively and run at 120 V for 1.5 h. After electrophoresis, the gel was fixed in a solution of 50% methanol, 15% acetic acid and 35% water with shaking (Heidolph Unimax 2010, Heidolph, Schwabach, Germany). The gel was further stained for 1h and destained until the background was clean before taking photos.

2.7. Statistics

Statistical analyses of glucosinolates in rapeseed meal after SSF with different microbes were carried out by using general linear models procedure in SAS (SAS, 1999). The results were subjected to one-way analysis of varinace, with microbe as the class variable. Significant difference ($P < 0.05$) were ranked by Duncan's multiple range method. The results are presented as mean ± standard error of mean (SEM).

3. Results

3.1. Effect of SSF on Glucosinolates and Peptide

The content of glucosinolates in rapeseed meal after SSF with five microbes is presented in Table 2. The results showed that rapeseed meal fermented by *S. cerevisiae* resulted in lowest content of

glucosinolates (32.8 mg/g, dry matter basis), which was significantly lower than the ones fermented by *A. oryzae* and *Trichoderma* sp. MAB-2010b. The highest content was in rapeseed meal fermented by the *Trichoderma* sp. MAB-2010b (67.5 mg/g, dry matter basis), followed by the *A. oryzae* (47.6 mg/g, dry matter basis).

Table 2. Content of glucosinolates in rapeseed meal after solid state fermentation with different microbes

Rapeseed meal fermented by	Glucosinolates ($\mu\text{mol/g}$ dry matter)
<i>Aspergillus oryzae</i>	47.6 ± 2.0^b
<i>Aspergillus niger</i>	39.2 ± 1.8^{bc}
<i>Penicillium purpurogenum</i>	42.6 ± 3.7^{bc}
<i>Trichoderma</i> sp. MAB-2010b	67.4 ± 3.5^a
<i>Saccharomyces cerevisiae</i>	32.8 ± 3.6^c

Glucosinolates in raw rapeseed meal was $133.7 \mu\text{mol/g}$ dry matter;

Values are expressed as mean \pm SEM. Different letters indicate significant differences ($P < 0.05$).

The peptides in rapeseed meal before and after SSF are presented in Figure 1. The results showed that SSFs with *A. oryzae*, *A. niger*, *P. purpurogenum* and *Trichoderma* sp. MAB-2010b did not affect the peptide size of rapeseed meal (lane B to E). However, SSF with *S. cerevisiae* affected the peptide size. The peptide with size of around 20 kDa was clearly increased in rapeseed meal fermented with *S. cerevisiae* (lane F). The same size peptide was not increased in rapeseed meal fermented by other microbes.

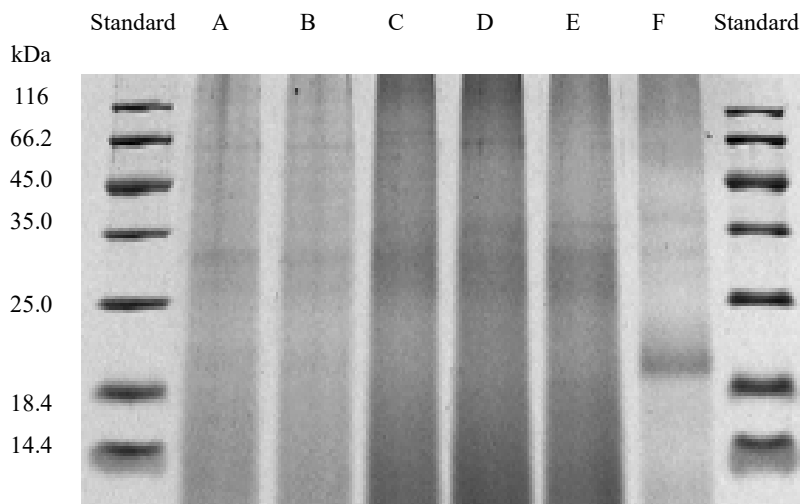


Figure 1. SDS-PAGE of rapeseed meal before and after solid state fermentation with different microbes

Lane A represents rapeseed meal without solid state fermentation;

Lane B represents rapeseed meal fermented by *Aspergillus oryzae*;

Lane C represents rapeseed meal fermented by *Aspergillus niger*;

Lane D represents rapeseed meal fermented by *Penicillium purpurogenum*;

Lane E represents rapeseed meal fermented by *Trichoderma* sp. MAB-2010b;

Lane F represents rapeseed meal fermented by *Saccharomyces cerevisiae*.

3.2. Optimization of SSF with *S. cerevisiae*

In general, SSF with *S. cerevisiae* showed the high level in degradation of glucosinolates and influence of peptide size. Thus, the optimization process was just carried out for SSF with *S. cerevisiae*. The design matrix of the variables in coded units is depicted in Table 3 along with the predicted and experimental values of the dependent variable. The quadratic model, representing the content of glucosinolates (Y) as a response of substrate to water ratio (A), inoculum amount (B) and duration (C), was expressed by the Equation (2). In order to obtain a better fit model, some of the statistically insignificant terms ($P > 0.05$), including B^2 and C^2 , were taken away from the model.

$$Y=14.59-37.40A-1.76B+11.22C+7.35AB-20.07AC+0.76BC+39.49A^2 \quad (2)$$

The statistical significance of Equation (2) was determined by Fisher's F-test. The ANOVA of the quadratic regression model (Table 4) demonstrated that Equation (2) was an extremely significant model at 99% confidence level based on P value ($P < 0.01$). The substrate to water ratio ($P < 0.01$), duration ($P < 0.05$) and their interaction ($P < 0.05$) significantly affected the content of glucosinolates in rapeseed meal. However, the inoculum amount, as well as the interaction between the inoculum amount and other two factors did not influence the content of glucosinolates.

Table 3. Box-Behnken design matrix with the experimental and predicted values of glucosinolates content in *Saccharomyces cerevisiae* fermented rapeseed meal

Run	A:Substrate to water ratio	B:Inoculum amount (mL)	C:Duration (h)	Glucosinolates ($\mu\text{mol/g}$ dry matter)	
				Actual value (n=3)	Predicted value
1	1	0.5	48	15.19 \pm 2.56	5.89
2	0.5	1	48	46.25 \pm 5.85	60.19
3	1.5	1	48	11.31 \pm 1.01	25.53
4	1	1.5	48	10.89 \pm 2.05	0.85
5	0.5	0.5	72	104.47 \pm 7.03	100.59
6	1.5	0.5	72	15.26 \pm 3.17	11.10
7	1	1	72	16.36 \pm 2.07	14.59
8	1	1	72	16.10 \pm 0.21	14.59
9	1	1	72	14.33 \pm 0.46	14.59
10	0.5	1.5	72	85.51 \pm 4.91	82.37
11	1.5	1.5	72	25.70 \pm 3.95	22.27
12	1	0.5	96	15.27 \pm 0.42	26.82
13	0.5	1	96	129.69 \pm 6.25	122.77
14	1.5	1	96	14.47 \pm 2.28	7.84
15	1	1.5	96	14.00 \pm 0.94	24.81

Values are expressed as mean \pm SEM.

Table 4. ANOVA of regression

Source	Sum of squares	df	Mean square	F-value	P>F
Model	19872	7	2839	20.2	< 0.01**
A (Substrate to water ratio)	11188	1	11188	79.5	< 0.01**
B (Inoculum amount)	25	1	25	0.2	0.69
C (Duration)	1008	1	1008	7.2	0.03*
AB	216	1	216	1.5	0.26
AC	1611	1	1611	11.5	0.01*
BC	2.3	1	2.3	0.02	0.90
A ²	5822	1	5822	41.4	< 0.01**
Residual	985	7	141		
Lack of fit	983	5	197	160.8	0.06
Pure error	2.5	2	1.2		
Corrected total	20857	14			

*Significant difference; **Extremely significant difference.

The graphs, representing the regression of Eq. (2), were obtained using the Design Expert (Figure 2). The graphs were generated for the pair-wise combination of the three variables, while inoculum amount was kept at the optimal level (1.5 mL) for the lowest content of glucosinolates.

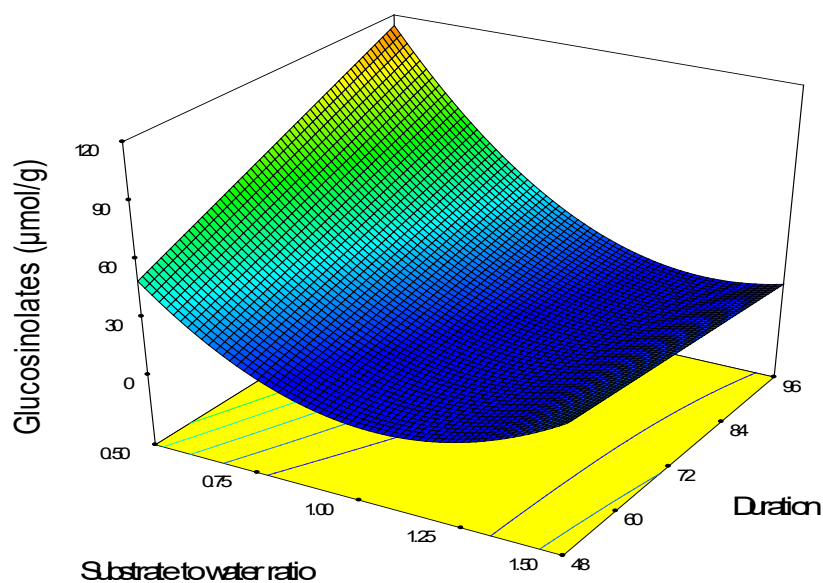


Figure 2. Response surface 3D plot of glucosinolates in rapeseed meal during solid state fermentation with *Saccharomyces cerevisiae*, showing the effects of substrate to water ratio and duration (Inoculum amount was 1 mL that was the optimal level)

The optimal values of the three factors during fermentation for obtaining the predicted minimum content of glucosinolates (0.22 $\mu\text{mol/g}$, dry matter basis) derived from response surface were delineated as the followings: 1.0 of substrate to water ratio, 1.5 mL of inoculum amount and 48 h of duration.

3.3. Validation of Optimal Conditions of SSF

An additional SSF experiment was performed with the optimal substrate to water ratio, inoculum amount and duration to confirm the optimal conditions. The experiment was performed in triplicate. The mean content of glucosinolates in fermented rapeseed meal was 0.46 $\mu\text{mol/g}$ dry matter, which was close to the estimated value (0.22 $\mu\text{mol/g}$ dry matter basis). The result thus corroborated the validity of the response surface model.

4. Discussion

The total glucosinolates in rapeseed meal were efficiently reduced by SSF with five selected microbes, in keeping with previous findings (Bau *et al.*, 1994; Smits, Knol, & Bol, 1993; Vig & Walia, 2001; Wang *et al.*, 2012). The degradation of glucosinolates in fermentation may be due to utilization of glucose and sulphur moieties of these compounds by microbial enzymes (Tripathi & Mishra, 2007). In the present study, the molds reduced the content of glucosinolates from 133.7 to 39.2, 42.6, 47.6 and 67.4 $\mu\text{mol/g}$ (dry matter basis). Yeast (*S. cerevisiae*) exhibited higher efficiency in degradation of glucosinolates, and reduced the content of glucosinolates from 133.7 to 32.8 $\mu\text{mol/g}$ (dry matter basis). However, the results from Wang *et al.* (2012) showed that yeast had a lower ability to reduce glucosinolates compared to molds in general. Some yeast fermentation even elevated the content of glucosinolates in rapeseed meal. The disagreement between that and current experiment may be a result of the different types of yeast and mold used.

The fermentation with *S. cerevisiae* increased the amount of small peptide with size of around 20 kDa in rapeseed meal. The increase may be due to partial digestion of peptides with size larger than 20 kDa by proteases secreted by the yeast. The types of peptide with 20kDa generated in the present experiment were unclear. It will be interesting if we could identify them in the following experiment. Secretion of proteases in many types of yeast has been proved, including *S. cerevisiae* (Ogrydziak, 1993). However, some previous studies also concluded that the molds had the capability of secreting proteases. The study conducted by Sardjono, Zhu, and Knol (1998) showed that *A. oryzae* obtained highest protease activity after a 48-hour fermentation. In addition, Hong *et al.* (2004) demonstrated that *A. oryzae* fermentation reduced the peptide size of soybean and soybean meal. Sandhya, Sumantha, Szakacs, and Pandey (2005) compared effect of SSF and submerged fermentation on production of proteases in *A. oryzae*, and proved the superiority of SSF over submerged fermentation. The capability in protease production of *A. niger* also was confirmed (Lopes *et al.*, 2011). In contrast, Rodarte, Dias, Vilela, and Schwan (2011) demonstrated that *P. purpurogenum* did not show protease activities by hydrolysis of casein. There was no study concerning the protease production of *Trichoderma* sp. MAB-2010b. However, Kredics *et al.* (2005) reviewed the extracellular proteases of *Trichoderma* species. It may indicate that most of *Trichoderma* species secrete extracellular proteases, and thus, may be able to digest peptide with large size to some extent.

S. cerevisiae showed the higher capability in digestion of peptides compared to the other microbes in the present study. This may be ascribed to that the efficiency of protease secretion and protease activity was different in SSF. Unfortunately, the protease activity was not detected in the present study. Thus, the protease activity of those microbes should be detected in the following experiment. In addition, the protease activity was affected by the different environmental conditions. Braaksma, Smilde, van der Werf, and Punt (2009) concluded that culture pH and nitrogen

concentration strongly affected extracellular protease activities in fermentation of *A. niger*. The initial condition of SSF was set to be the same in the present study. However, the production of organic acids may be occurred during fermentation. Therefore, the pH was varied subsequently during fermentation, and was not the same amongst fermentations with different microbes.

The optimal substrate to water ratio was 1.0 in SSF with *S. cerevisiae*. The moisture content of untreated rapeseed meal used in current work was 10%. Thus, the 1.0 of substrate to water ratio was equal to 55% of moisture in the solid matrix. This level was lower than the suggested moisture (60-72%) for yeast (Rodriguez-Leon, Soccol, Pandey, & Rodriguez, 2007). This variance may be caused by different water activity, which is an important factor for solid matrix. The optimal inoculum amount was 1.5 mL, which was equal to 5% in basis of that the amount of rapeseed meal was 30g in fermentation. To our knowledge, the studies concerning rapeseed fermentation by yeast were limited. However, the molds have been tried in SSF with rapeseed meal as substrate. Gu *et al.* (2010) attempted to increase the quality of by mixed SSF with *A. oryzae* and *T. viride*. The optimal inoculum amount was 30% in that study, which was higher than that in the current study. The different inoculum amount may be due to the different type of microbes. In principle, the hyphae of *A. oryzae* and *T. viride* can grow on particle surfaces and penetrate intrate into the inter-particle spaces, thereby colonizing solid substrates rapidly than yeast (dos Santos, da Rosa, Dal'Boit, Mitchell, & Krieger, 2004). Therefore, the inoculum amount would be lower in fermentation with *A. oryzae* and *T. viride* than with *S. cerevisiae*. The duration significantly affected the content of glucosinolates. A previous experiment conducted by Gao, Wang, Zhu, and Qian (2013) also showed the significant effect of duration in SSF. The optimal duration was 48 h, which was the minimum in the setting range. It indicated that fermentation may reduce other components more rapidly than glucosinolates of rapeseed meal in the setting range. Thus, the relative content of glucosinolates was showed to be increased. However, the total amount of glucosinolates was not increased indeed.

In conclusion, SSF with *S. cerevisiae* degraded the glucosinolates more than SSF with other four microbes. In addition, the peptides were hydrolyzed by *S. cerevisiae* to a greater extent than other four strains. The optimal conditions derived from response surface methodology for *S. cerevisiae* SSF were: 1.0 of substrate to water ratio, 1 mL of inoculum amount, and 48 h of duration. The minimum content of glucosinolates was 0.46 mg/g dry matter. *S. cerevisiae* employed in the present study thus exhibit the potential use in large scale SSF for improving the nutritional quality of rapeseed meal.

Acknowledgements

This work was supported by Zhejiang Provincial Natural Science Foundation of China (Grant number: LY14C190009), Ningbo Science and Technology Bureau Foundation (Grant number: 2012B82016, 2014A610193) and Zhejiang Major Research Foundation (2012C12907).

References

- [1] Aluko, R. E., & McIntosh, T. (2001). Polypeptide profile and functional properties of defatted meals and protein isolates of canola seeds. *Journal of the Science of Food and Agriculture*, 81(4), 391-396.
- [2] Bau, H. M., Villaume, C., Lin, C. F., Evrard, J., Quemener, B., Nicolas, J. P., & Mejean, L. (1994). Effect of a solid-state fermentation using *Rhizopus oligosporus* sp.T-3 on elimination of antinutritional substances and modification of biochemical constituents of defatted rapeseed meal. *Journal of the Science of Food and Agriculture*, 65(3), 315-322.
- [3] Bell, J. M. (1984). Nutrients and toxicants in rapeseed meal: a review. *Journal of Animal Science*, 58(4), 996-1010.

-
- [4] Braaksma, M., Smilde, A. K., van der Werf M. J., & Punt, P. J. (2009). The effect of environmental conditions on extracellular protease activity in controlled fermentations of *Aspergillus niger*. *Microbiology*, 155, 3430-3439.
- [5] Burel, C., Boujard, T., Escaffre., A. M., Kaushik, S. J., Boeuf, G., Mol, K. A., Van der Geyten, S., & Kuhn, E. R. (2000). Dietary low-glucosinolate rapeseed meal affects thyroid status and nutrient utilization in rainbow trout (*Oncorhynchus mykiss*). *British Journal of Nutrition*, 83(6), 653-664.
- [6] Cheng, Z. Y., Ai, Q. H., Mai, K. S., Xu, W., Ma, H. M., Li, Y. & Zhang, J. M. (2010). Effects of dietary canola meal on growth performance, digestion and metabolism of Japanese seabass, *Lateolabrax japonicus*. *Aquaculture*, 305(1-4), 102-108.
- [7] dos Santos, M. M., da Rosa, A. S., Dal'Boit, S., Mitchell, D. A. & Krieger, N. (2004). Thermal denaturation: is solid-state fermentation really a good technology for the production of enzymes? *Bioresource Technology*, 93(3), 261-268.
- [8] El-Batal, A. I., & Karem, H. A. (2001). Phytase production and phytic acid reduction in rapeseed meal by *Aspergillus niger* during solid state fermentation. *Food Research International*, 34(8), 715-720.
- [9] Francis, G., Makkar, H. P. S., & Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, 199(3-4), 197-227.
- [10] Frias, J., Song, Y. S., Martinez-Villaluenga, C., De Mejia, E. G., & Vidal-Valverde, C. (2008). Immunoreactivity and amino acid content of fermented soybean products. *Journal of Agricultural and Food Chemistry*, 56(1), 99-105.
- [11] Gao, Y. L., Wang, C. S., Zhu, Q. H., & Qian, G. Y. (2013). Optimization of solid-state fermentation with *Lactobacillus brevis* and *Aspergillus oryzae* for trypsin inhibitor degradation in soybean meal. *Journal of Integrative Agriculture*, 12(5), 869-876.
- [12] Glencross, B., Hawkins, W., & Curnow, J. (2004a). Nutritional assessment of Australian canola meals. I. Evaluation of canola oil extraction method and meal processing conditions on the digestible value of canola meals fed to the red seabream (*Pagrus auratus*, Paulin). *Aquaculture Research*, 35(1), 15-24.
- [13] Glencross, B., Hawkins, W., & Curnow, J. (2004b). Nutritional assessment of Australian canola meals. II. Evaluation of the influence of the canola oil extraction method on the protein value of canola meals fed to the red seabream (*Pagrus auratus*, Paulin). *Aquaculture Research*, 35(1), 25-34.
- [14] Gong, W. J., Zhang, Y. P., Zhang, Y. J., Xu, G. R., Wei, X. J., & Lee, K. P. (2007). Optimization strategies for separation of sulfadiazines using Box-Behnken design by liquid chromatography and capillary electrophoresis. *Journal of Central South University of Technology*, 14(2), 196-201.
- [15] Gu, X. H., Zhang, Y. G., Zhao, G. M., Zhang, R. B., Zhang, F., & Zhu, J. H. (2010). Improvement of feeding value of rapeseed meal by mixed solid state fermentation. *Animal Husbandry and Feed Science*, 2(3), 12-14,17.
- [16] Hong, K. J., Lee, C. H., & Kim, S. W. (2004). *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *Journal of Medicinal Food*, 7(4), 430-435.

- [17] Kredics, L., Antal, Z., Szekeres, A., Hatvani, L., Manczinger, L., Vagvolgyi, C., & Nagy, E. (2005). Extracellular proteases of *Trichoderma* species. A review. *Acta Microbiologica Et Immunologica Hungarica*, 52(2), 169-184.
- [18] Lopes, F. C., Silva, L. A. D. E., Tichota, D. M., Daroit, D. J., Velho, R. V., Pereira, J. Q., Corrêa, A. P. F., & Brandelli, A. (2011). Production of proteolytic enzymes by a keratin-degrading *Aspergillus niger*. *Enzyme Research*, 2011. doi:10.4061/2011/487093
- [19] Mithen, R. F., Dekker, M., Verkerk, R., Rabot, S., & Johnson, I. T. (2000). The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. *Journal of the Science of Food and Agriculture*, 80(7), 967-984.
- [20] Nair, V. C., & Duvnjak, Z. (1991). Phytic acid content reduction in canola meal by various microorganisms in a solid state fermentation process. *Acta Biotechnologica*, 11(3), 211-218.
- [21] Ogrydziak, D. M. (1993). Yeast extracellular proteases. *Critical Review in Biotechnology*, 13(1), 1-55.
- [22] Rashad, M. M., Mahmoud, A. E., Abdou, H. M., & Nooman, M. U. (2011). Improvement of nutritional quality and antioxidant activities of yeast fermented soybean curd residue. *African Journal of Biotechnology*, 10(28), 5504-5513.
- [23] Rodarte, M. P., Dias, D. R., Vilela, D. M., & Schwan, R. F. (2011). Proteolytic activities of bacteria, yeasts and filamentous fungi isolated from coffee fruit (*Coffea arabica* L.). *Acta Scientiarum-Agronomy*, 33(3), 457-464.
- [24] Rodriguez-Leon, J. A., Soccol, C. R., Pandey, A., & Rodriguez, D. E. (2007). Factors affecting solid-state fermentation. In A. Pandey, C. R. Soccol, & C. Larroche (Eds.), *Current Developments in Solid-state Fermentation* (p.26-47). New Delhi, India: Asiatech Publishers, Inc.
- [25] Rozan, P., Villaum, C., Bau, H. M., Schwertz, A., Nicolas, J. P., & Mejean, L. (1996). Detoxication of rapeseed meal by *Rhizopus oligosporus* sp-T3: A first step towards rapeseed protein concentrate. *International Journal of Food Science and Technology*, 31(1), 85-90.
- [26] Sandhya, C., Sumantha, A., Szakacs, G., & Pandey, A. (2005). Comparative evaluation of neutral protease production by *Aspergillus oryzae* in submerged and solid-state fermentation. *Process Biochemistry*, 40(8), 2689-2694.
- [27] Sardjono, Zhu, Y., & Knol, W. (1998). Comparison of fermentation profiles between lupine and soybean by *Aspergillus oryzae* and *Aspergillus sojae* in solid-state culture systems. *Journal of Agricultural and Food Chemistry*, 46(8), 3376-3380.
- [28] SAS. (1999). *SAS/STAT User's Guide Version 8*. Cary, NC.: SAS Publishing.
- [29] Smits, J. P., Knol, W., & Bol, J. (1993). Glucosinolate degradation by *Aspergillus clavatus* and *Fusarium oxysporum* in liquid and solid-state fermentation. *Applied Microbiology and Biotechnology*, 38(5), 696-701.
- [30] Tanii, H., Takayasu, T., Higashi, T., Leng, S., & Saijoh, K. (2004). Allylnitrile: generation from cruciferous vegetables and behavioral effects on mice of repeated exposure. *Food and Chemical Toxicology*, 42(3), 453-458.
- [31] Tripathi, M. K., & Mishra, A. S., (2007). Glucosinolates in animal nutrition: A review. *Animal Feed Science and Technology*, 132(1-2), 1-27.
- [32] USDA. (2013). *Oilseeds: World Markets and Trade* (Circular Series FOP 08-13). Washington, DC.: United States Department of Agriculture (USDA). Retrieved from

<http://usda.mannlib.cornell.edu/usda/fas/oilseed-trade//2010s/2013/oilseed-trade-08-12-2013.pdf>.

- [33] Vig, A. P., & Walia, A. (2001). Beneficial effects of *Rhizopus oligosporus* fermentation on reduction of glucosinolates, fibre and phytic acid in rapeseed (*Brassica napus*) meal. *Bioresource Technology*, 78(3), 309-312.
- [34] Vohra, A., & Satyanarayana, T. (2002). Statistical optimization of medium components by response surface methodology to enhance phytase production by *Pichia anomala*. *Process Biochemistry*, 37(9), 999-1004.
- [35] Wallig, M. A., Belyea, R. L., & Tumbleson, M. E. (2002). Effect of pelleting on glucosinolate content of Crambe meal. *Animal Feed Science and Technology*, 99(1-4), 205-214.
- [36] Wang, N. (2009). A quantitative analysis method of glucosinolates in rapeseed meal. *Science and Technology of Qinghai Agriculture and Forestry*, 3, 58-59.
- [37] Wang, X., Jin, Q., Wang, T., Huang, J., Xia, Y., Yao, L., & Wang, X. (2012). Screening of glucosinolate-degrading strains and its application in improving the quality of rapeseed meal. *Annals of Microbiology*, 62(3), 1013-1020.
- [38] Yigzaw, Y., Gorton, L., Solomon, T., & Akalu, G. (2004). Fermentation of seeds of teff (*Eragrostis teff*), grass-pea (*Lathyrus sativus*), and their mixtures: aspects of nutrition and food safety. *Journal of Agricultural and Food Chemistry*, 52(5), 1163-1169.

Copyrights



Copyright for this article is retained by the author(s), with first publication rights granted to the journal. This is an open-access article distributed under the terms and conditions of the [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

