DOI:10.1021/jf9044588

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Effects of Storage Condition Factors on Fungal Invasion of Radix Ophiopogonis

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Fungal invasion is a main factor leading to the loss of postharvest herbs during storage. To prevent fungal invasion and clarify the favorable conditions for the stability of herbs during the storage period, uniform design and three-dimensional response surfaces were applied to investigate the relationship of the mildew degree of Radix Ophiopogonis induced by prevalent fungal isolates and the storage factors including air relative humidity (X_1), temperature (X_2), and moisture content (X_3), in laboratory studies. Mildew degree was evaluated by ergosterol assay of mold isolates through a high-performance liquid chromatography−diode array detector (HPLC-DAD) technique. As a result, storage conditions, which satisfied the following correlation equations established by the relationships between the storage factors and the biomass of mildew (Y), could effectively prevent fungal spoilage: $Y = -0.2484084028 + 0.00030711966572 \times X_3 \times X_3 + 0.00018881361186 \times X_1 \times X_2 - 0.00029473040679 \times X_2 \times X_3 \leq 0$; $Y = -0.2697457586 + 0.004127756022 \times X_1 - 0.000015977780492 \times X_1 \times X_1 + 0.00021906984606 \times X_2 \times X_3 \leq 0$; $Y = -0.325655811 + 0.015464432582 \times X_2 + 0.00004779394354 \times X_1 \times X_1 - 0.00021743815482 \times X_2 \times X_2 \leq 0$. This compositive methodology might be useful to predict the occurrence of fungal invasion.

KEYWORDS: Fungal invasion; storage factors; uniform design; Radix Ophiopogonis; condition control

INTRODUCTION

Herbs are cultivated as normal agricultural products in Genuine Producing Areas (GPA) in China. Before circulation into the application fields, they are usually stored in warehouses after some simple processing, such as open-air drying and sweating. However, fungal infection, together with the unstable conditions of the warehouse, may partially account not only for unpleasant appearance and odor but also for the development of mold, which leads to quality reduction and economic loss of these postharvest agricultural products during storage (*1*). Some secondary metabolites produced by fungi, such as mycotoxins (*2*), are proven to be toxic to human body (*3*).

To reduce human exposure to fungi and ensure the safety of agricultural products, prevention and control programs are necessary. Although numerous studies have already been reported in this field, most of them were focused on the methods to inhibit pathogenic fungi (*4*) and inactivate microorganisms on the surfaces of food material (*5*−*7*). The above methods included the use of chemical disinfectants such as hydrogen peroxide and chlorine and physical methods such as ultraviolet irradiation, pasteurization, and microwave processing. However, chemical disinfectants, such as chlorine, could combine or react with organic materials to produce harmful byproducts such as chloramines and trihalomethanes, which could bring harm to human health and the environment (*8*, *9*). Heating processes induced by pasteurization and microwave processing, especially when the temperature exceeded 70 °C, could cause unacceptable organoleptic degradation of herb medicines, including texture loss, discoloration, and odor (*10*). Meanwhile, it is still unclear whether the chemical or physical effects of the above-mentioned methods will affect the pharmacodynamic action of herbs. Therefore, the strategy to inhibit fungal invasion by controlling influencing factors will be of great significance and be more desirable in developing countries from an economic viewpoint.

Herbal medicines, which are rich in saccharide and water, are highly susceptible to fungal invasion. A good case in point is Radix Ophiopogonis, having a saccharide content of up to 40% (*11*) and a water content in fresh material of about 50%. In this study, Radix Ophiopogonis was taken as an example to investigate the effect of the storage condition on mildew degree of agriculture products.

Radix Ophiopogonis is the tuber roots of *Ophiopogon japonicus* (Thunb.) Ker-Gawl. The plant *Ophiopogon j.*, first recorded in "Shengnong Bencaojing", was primarily employed in the treatment of latent heat in the lungs caused by "yin"-asthenia, fever in

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 Published on Web 04/16/2010

consumptive disease or general debility, and dehydration in febrile disease as well as dry mouth (*12*). Nowadays, Radix Ophiopogonis is considered as a traditional agricultural product with both food and medical functions (*13*) and is not only widely used as a herbal medicine for acute and chronic inflammation and cardiovascular diseases (*12*) but also consumed as a popular soup stock and dry fruit in Chinese daily life.

The degree of fungal invasion on herbal medicine is greatly influenced by environmental and endogenous factors (*14*). Water content, relative humidity, and temperature are the most important factors influencing mold growth during the storage of Radix Ophiopogonis. Inappropriate storage conditions will also increase the number of contaminating molds because these organisms grow more rapidly in certain conditions (*15*). To understand the effects of these factors and their interactions and to find a suitable storage condition, the relationship between influencing factors and mildew degree should be revealed. In this case, a rational experimental design and suitable accelerated experiment model is very crucial for the determination of optimal experimental conditions as well as the prediction of fungal growth trends in preservation.

The aim of the present work was to investigate the prevalent molds that induced mildew and the influence of the storage factors on the growth of mold strains in Radix Ophiopogonis. The relationship between mildew degree and storage factors was established by statistical methods. The compositive methodology that integrated uniform design with three-dimensional response surfaces might provide valuable insights into the investigation of optimal storage conditions. With the methodology, fungal invasion could be effectively restrained simply by controlling the storage factors.

MATERIALS AND METHODS

Isolation of External Infected Mold. Radix Ophiopogonis was collected from a production base in Ya'an of Sichuan province. Samples were washed with sterile water, and serial dilutions of the washing solutions were placed on the following 10 cm Petri dish media as described by Samson et al. (*16*): potato dextrose agar (PDA), consisting of potato extract (200 g), dextrose (15 g), KH_2PO_4 (3 g), $MgSO_4$ (0.73 g), agar (20 g), and H_2O (1000 mL); Czapek−Dox medium, consisting of sucrose (30 g), $NaNO_3$ (2 g), KCl (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), K_2HPO_4 (1 g), agar (15 g), and H_2O (1000 mL); and Gause's no. 1 synthetic medium, consisting of soluble starch (20 g), K_2HPO_4 (0.5 g), NaCl (0.5 g), KNO_3 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), agar (20 g), and H_2O (1000 mL) (*17*).

The inoculated agar media were incubated at 28 ± 1 °C until sporulation was complete. Aerial mycelium of the mold colonies was streak-inoculated onto agar medium and incubated for 7 days in the dark at 28 ± 1 °C. Mold isolates were purified on PDA and further subcultured for microscopic examination and identification.

Isolation of Endogenetic Fungi. The surface of Radix Ophiopogonis was sterilized with a 2% NaClO solution for 10 min, followed by 75% ethanol for 1 min, and then washed with sterile water three times (*18*). The sterile tuber roots were cut into 0.2 cm × 0.2 cm pieces and cultured on PDA and Czapek medium at 28 ± 1 °C for 14 days. Visible mold colonies were isolated and purified.

Selection of Prevalent Molds. Fresh Radix Ophiopogonis samples were wiped with 75% ethanol solution and further disinfected by ultraviolet irradiation for 30 min. All of the mold isolates were cultured respectively on PDA at 28 ± 1 °C for 7 days and cut into 1.5 cm pieces by a puncher. Then the pieces were transferred into centrifuge tubes with 2 mL of sterilized 0.5% Tween-20 solution and vortexed for 1 min; then 1 mL of suspension from each isolate was separately inoculated on the surface of Radix Ophiopogonis in culture dishes. Inoculated dishes were cultured at 28 ± 1 °C for 14 days.

Mold Identification. The prevalent mold isolates were identified to species level by their macroscopic and microscopic morphological characters (*16*). Macroscopic features include colony color, texture, diameter, and the production of diffusible pigments and exudates, whereas microscopic characters involve conidia and conidiophore arrangements. All of the isolates were identified according to the guidelines of Samson et al. (*16*) and Pitt and Hocking (*17*). Molecular identification by sequencing the internal transcribed spacer (ITS) region of fungal DNA was performed to verify the morphological identification of the dominant mold isolates.

Preparation of Sample Solutions. The mold isolates were incubated on 15 mL of PDA medium in Petri dishes at 25 °C for 7 days. After the inoculated agar medium had been melted in a water bath, the fungi were filtered and washed with water and dried at 60 °C until a constant weight was obtained. Dry fungi were pulverized into homogeneous powder (granularity ≤ 0.25 mm).

Accurately weighed fungi were introduced into the flask and refluxed with the mixture solvent of 20 mL of methanol, 10 mL of 20% NaOH, 4 mL of 95% ethanol, and 20 mL of normal heptane at 85 °C for 1.5 h. After cooling at room temperature (25 °C), the heptane layer was separated from the demixed solution and washed with 20 mL of water three times. Ten milliliters of the upper layer (heptane) was precisely sampled and evaporated under vacuum, and the residue was dissolved with methanol and diluted to 1 mL. All reagents and solvents used for extraction were of analytical reagent grade and were purchased from Nanjing Chemical Regent Corp., Jiangsu, China.

Determination of Ergosterol Content in Mold Isolates. Strains on Petri plate were taken and analyzed for ergosterol content. After filtration through a 0.45 μm acetate filter, an aliquot of 10 μL of sample solution was injected into the HPLC. The HPLC equipment used was an Agilent 1100 series consisting of a binary pump, autosampler, thermostated column compartment, and a DAD system. A Lichrospher C_{18} column (5 μm i.d., 250 mm × 4.6 mm, Hanbon Science and Technology, Jiangsu, China) was used for all chromatographic separations. The column was maintained at 25 °C, and the eluent was methanol at a flow rate of 1 mL/min. The detection wavelength was set at 282 nm. The above-described method for ergosterol analysis was completely validated.

The standard curve of ergosterol, plotted using peak area versus concentration, was linear in the range from 0.002 to 0.2 μg. The regression equation was found to be $y = 0.00006x - 0.0015$ ($R^2 = 0.9996$). The average recovery of ergosterol was 97.50%.

All of the reagents and solvents used for chromatography were of HPLC grade. Ergosterol reference standard was purchased from Aladdin Reagent Database Inc.

Influence of Storage Conditions on the Development of Prevalent Molds on Radix Ophiopogonis: Ergosterol Assay of Fungus-Infected Tuber Roots. The correlation coefficient between ergosterol and colony diameters was constant for each of the strains (*19*). According to this conclusion, colonies of the mold isolates were perforated into identical size by a puncher (diameter = 1.5 cm) and transferred into centrifuge tubes with 2 mL of sterile 0.5% Tween-20 solutions. The solutions were prepared into suspension by vortexing the tubes for 1 min. Five grams of fresh Radix Ophiopogonis plated in an aseptic Petri dish was wiped with 75% EtOH and ultraviolet sterilized for 30 min. One milliliter of suspension was sprayed on the surface of tuber roots. The inoculated Petri dishes were cultured in a mold incubator (MJX-128) (Ningbo Jiangnan Instrument Factory, China) under the conditions arranged by uniform design below. All of the samples were dried to prepare for ergosterol assay according to the methods mentioned above.

Experimental Design. *Selection of Factor Level in Uniform Design.* In this paper, experimental runs were conducted by uniform design. The factors were chosen to simulate the climate conditions of different areas of Mainland China and embody the characteristics of Radix Ophiopogonis, which were directly related to the susceptibility of fungal spoilage. Levels of the factors were selected on the basis of the requirement of uniform design.

There were five levels for each experimental factor and a $U_5(5^3)$ uniform design was selected. The factors and levels of the tests are shown in **Table 1**. The effects of air relative humidity (% RH, X_1), temperature (°C, X_2), and moisture content (%, X_3) on the biomass of fungi were examined.

Algorithm. The experimental data of uniform design were analyzed by the quadratic polynomial stepwise regression method. The generalized model proposed for the analysis can be described by the equation

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i \le j}^{k} \beta_{ij} x_i x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \varepsilon$$

where k is the number of variables, Y is the dependent variable, β_0 is the constant coefficient, β_i is the linear coefficient, β_{ij} is the interaction coefficient, β_{jj} is the quadratic coefficient, and x_i and x_j are the reality values of independent variables.

RESULTS AND DISCUSSION

Mold Isolation and Selection of Prevalent Molds. In total, 21 molds were isolated from the surface of dry Radix Ophiopogonis samples, whereas 4 were found as endogenetic fungi. However, only three molds could be observed on the surface of disinfected fresh Radix Ophiopogonis after inoculation and cultivation at 28 ± 1 °C for 14 days. The three molds were isolated from the surface of the tuber roots, so fungi from the external environment were the most common cause of mold development. This may mainly result from frequent contact with soil, insects, and humans during growing and harvesting process.

The molds from the surface of naturally mildewed Radix Ophiopogonis were also separated and purified. Four strains were isolated, and three of them were identified to be the same as the three molds growing on the surface of disinfected fresh Radix Ophiopogonis after inoculation. As a result, the three molds could be considered as prevalent molds that caused the mildew of Radix Ophiopogonis. According to the macroscopic and microscopic morphological characters (*16*, *17*) and ITS region sequences, they were identified as *Aspergillus flavus*, *Aspergillus tubingensis*, and *Gibberella avenacea*.

Content of Ergosterol in Mold Isolates. The effects of storage conditions on the mildew degree of Radix Ophiopogonis were measured by mold growth, which could be indicated by fungal biomass. However, unlike fluid cultivation, fungal biomass cannot be quantitatively separated from the solid medium, so direct measurement is not feasible (*20*). Colony diameter is the most common index to assess mold growth in solid substrates, but it can hardly be applied to real food substrates, especially on irregular surfaces (*21*). Marín's research (*22*) showed that total fungal biomass was correlated to dry weight for the same strains. Ergosterol is commonly distributed in almost all of the fungi (*23*), and total ergosterol content is strongly associated with the actual amount of fungal mass (*24*, *25*). As a fungal biochemical marker, ergosterol content is hardly affected by tissue age (*26*) and can be considered as a good indicator and reliable measurement of fungal dry weight (*19*), which has been used to evaluate mold contamination in cereals and other food products (*27*, *28*). In our study, ergosterol was chosen as an indicator of mildew degree to investigate the impact of storage condition factors.

Table 1. Factor-Level List for Uniform Design

factor	air relative humidity (% RH)	temperature (°C)	moisture content (%)
1	45	15	12
2	58	21	18
3	71	27	30
4	83	33	40
5	95	40	50

A specificity test was carried out before the analysis of ergosterol. As shown in **Figure 1**, no chromatographic peak of ergosterol could be observed in the chromatogram of Radix Ophiopogonis, which indicated that Radix Ophiopogonis had no interference with the determination of ergosterol.

Liquid−liquid extraction (LLE) is a conventional method for the extraction of ergosterol. Fungus samples are first hydrolyzed by methanolic KOH and then extracted by low-polarity solvent (*29*). However, poor reproducibility is a common problem in the LLE method. In addition, it is a time-consuming procedure and needs large amounts of toxic solvents (*30*). To obtain better accuracy, heptane was added into the flask before hydrolyzation. In this way, the extraction rate of ergosterol could be improved.

The ergosterol content in dry mycelium was stable when cultured for 7 days at 25 °C on a certain amount of solid culture medium (15 mL of PDA). As shown in **Figure 2**, the contents of ergosterol in *A. flavus*, *A. tubingensis*, and *G. avenacea* were 0.07872, 0.1264, and 0.07887% (w/w), respectively.

Influence of Storage Conditions on the of Growth of Prevalent Molds on Radix Ophiopogonis. The surface of Radix Ophiopogonis should be sterilized to avoid interferences from other microorganisms before inoculation. To keep water content at a constant level and prevent residual chemical disinfectants from inhibiting the growth of inoculated fungi, a 75% ethanol solution and ultraviolet irradiation were employed in the process.

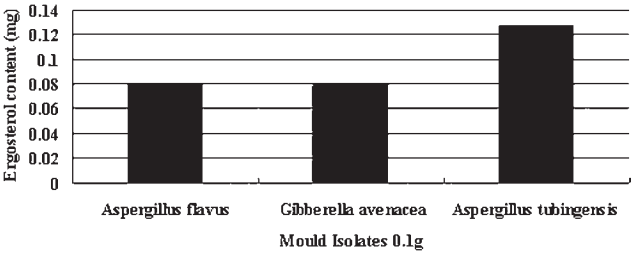

Figure 2. Ergosterol content in mold isolates.

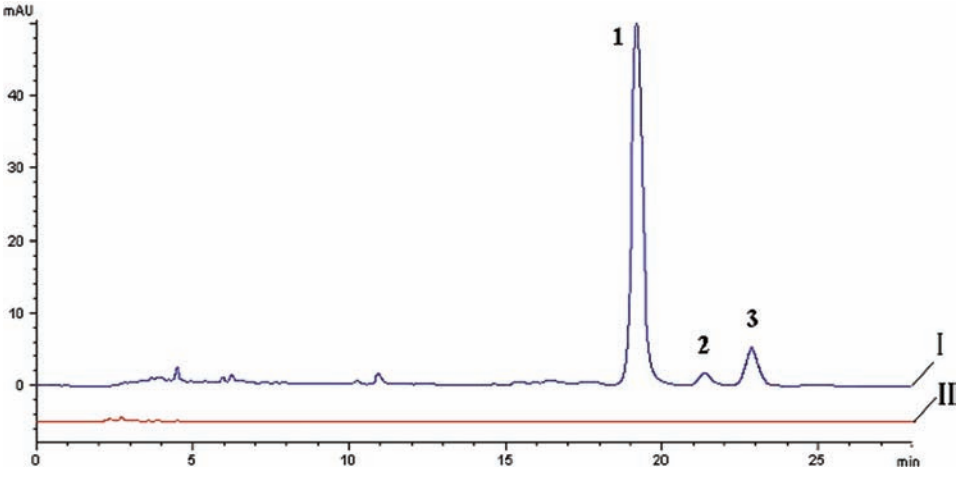

Figure 1. Specificity test of ergosterol (peak 1 is the peak of ergoserol): I, HPLC-DAD chromatogram of Radix Ophiopogonis substrate; II, HPLC-DAD chromatogram of *A. flavus*-invaded Radix Ophiopogonis.

Table 2. Uniform Design Factors and Results[a]

no.	air relative humidity (X_1, % RH)	temperature (X_2, °C)	moisture content (X_3, %)	*A. flavus*[b] (mg)	*G. avenacea*[b] (mg)	*A. tubingensis*[b] (mg)
1	45 (1)	21 (2)	40 (4)	0.1662	0.0679	0.0049
2	58 (2)	33 (4)	30 (3)	0.0916	0.1319	0.1044
3	71 (3)	15 (1)	18 (2)	0.0035	0.0100	0.0959
4	83 (4)	27 (3)	12 (1)	0.1184	0.0208	0.2625
5	95 (5)	40 (5)	50 (5)	0.6522	0.4166	0.3783

[a] Values represent means for data from three experiments; [b] Milligrams of fungi per 5 g of *Ophiopogon j.* tuber roots.

Ergosterol contents in 5 g of Radix Ophiopogonis were detected by HPLC and then converted into biomass of the three prevalent fungi on the basis of the results mentioned above.

In this work, mildew degree was investigated by uniform design optimization. Uniform design is a space-filling design, which can reflect the major characteristics of the experimental system and enormously reduce the amount of experiments (*31*, *32*). Due to the growth period of fungi and five levels for each factor, hundreds of experimental runs are needed, which require a tremendous amount of time. Therefore, accelerated experiment and uniform design method were employed to explore the relationships between response and factors with a minimum number of experiments. Accelerated experiment was carried out by high concentration of fungal suspension solution (incubated on the surface of Radix Ophiopogonis) and persistent air relative humidity and stable temperature provided by a mold incubator connected to a humidifier. With respect to moisture content, samples of Radix Ophiopogonis collected from production base in Ya'an, Sichuan province, were air-cured until the moisture content was <12% (the water content of fresh tuber roots was 50%, whereas that of the air-cured was 11.40%). Sterile water was insufflated on the surface of Radix Ophiopogonis in an airtight vessel to meet the requirement of uniform design. The results of the experiment are shown in **Table 2**.

Analysis of the Results of Uniform Design. The experimental data were analyzed by the quadratic polynomial stepwise regression method. The relationships between the tested factors and the biomass of mildew could be described by the following general equations.

Relationships between the tested factors and the biomass of *A. flavus*:

$$Y = -0.2484084028 + 0.00030711966572 \times X_3 \times X_3 + 0.00018881361186 \times X_1 \times X_2 - 0.00029473040679 \times X_2 \times X_3 \quad (1)$$

Relationships between the tested factors and the biomass of *G. avenacea*:

$$Y = -0.2697457586 + 0.004127756022 \times X_1 - 0.000015977780492 \times X_1 \times X_1 + 0.00021906984606 \times X_2 \times X_3 \quad (2)$$

Relationships between the tested factors and the biomass of *A. tubingensis*:

$$Y = -0.325655811 + 0.015464432582 \times X_2 + 0.00004779394354 \times X_1 \times X_1 - 0.00021743815482 \times X_2 \times X_2 \quad (3)$$

The correlation coefficient of the equation, F value, significance level, residual standard deviation, and correlation coefficient after adjustment of the three equations are listed in **Table 3**. The results of significance t test (namely, p value) for each variable of the three equations are shown in **Tables 4**, **5**, and **6**, respectively.

Table 3. Correlation Coefficient of the Three Equations

correlation coefficient	eq 1	eq 2	eq 3
correlation coefficient of the equation, R	0.9992	1	0.9997
F value	198.6718	8203.736	565.042
significance level, p	0.0521	0.0081	0.0309
residual standard deviation, S	0.021	0.0021	0.0072
correlation coefficient after adjustment, R_a	0.9966	0.9999	0.9988

Table 4. Significance t Test (Namely, p Value) for Each Variable of Equation 1

factor	partial correlation	t test	significance level, p value
$r(y, X_3 \times X_3)$	0.9905	7.2151	0.0187
$r(y, X_1 \times X_2)$	0.9948	9.7404	0.0104
$r(y, X_2 \times X_3)$	−0.9691	3.9312	0.0590

Table 5. Significance t Test (Namely, p Value) for Each Variable of Equation 2

factor	partial correlation	t test	significance level, p value
$r(y, X_3 \times X_3)$	0.9830	5.3496	0.0332
$r(y, X_1 \times X_2)$	−0.9425	2.8189	0.1062
$r(y, X_1 \times X_3)$	0.9999	87.3051	0.0001

Table 6. Significance t Test (Namely, p Value) for Each Variable of Equation 3

factor	partial correlation	t test	significance level, p value
$r(y, X_2)$	−0.9813	5.0919	0.0365
$r(y, X_1 \times X_1)$	0.9993	26.3117	0.0014
$r(y, X_2 \times X_2)$	−0.9670	3.7975	0.0629

To reveal the combined action of storage factors on the growth of the three fungi and find the appropriate storage conditions, an intuitionistic relationship graph was necessary. In the Chinese Pharmacopoeia, the water content of Radix Ophiopogonis should not be higher than 18% (*33*). On the basis of this regulation, X_3 in eqs 1, 2, and 3 was substituted by 18, and the following equations were obtained:

$$Y = -0.1489016311 + 0.00018881361186 \times X_1 \times X_2 - 0.005305147322 \times X_2 \quad (4)$$

$$Y = -0.2697457586 + 0.004127756022 \times X_1 - 0.000015977780492 \times X_1 \times X_1 + 0.003943257229 \times X_2 \quad (5)$$

$$Y = -0.325655811 + 0.015464432582 \times X_2 + 0.00004779394354 \times X_1 \times X_1 - 0.00021743815482 \times X_2 \times X_2 \quad (6)$$

MATLAB (version 7.0.1) software (Mathworks, Inc., Natick, MA) was used to generate the three-dimensional response surfaces

of fungal biomass, air relative humidity, and temperature, according to the three equations (**Figure 3**).

On the basis of the three-dimensional response surfaces, ergosterol could not be detected when Y was ≤0. In other words, it could be considered that no fungi invaded Radix Ophiopogonis when X_1 and X_2 satisfied the following inequations:

$$(0.001268043 \times X_1 - 0.035628537) \times X_2 < 1 \quad (7)$$

$$0.015302395 \times X_1 - 0.0000592327 \times X_1 \times X_1 + 0.0146184216 \times X_2 < 1 \quad (8)$$

$$0.0474870463 \times X_2 + 0.0001467621 \times X_1 \times X_1 - 0.0006676932 \times X_2 \times X_2 < 1 \quad (9)$$

Verification of the Results. Verification experiments were carried out to substantiate the speculation. The validated results are listed in **Table 7**. No ergosterol was detected in 7 days when the air relative humidity was up to 54% RH, with temperature and moisture content constant at 15 °C and 18%, respectively. At the same time, as shown in **Figure 4** (**1a**, **2a**, and **3a**), no fungal mycelia could be observed on the surface of Radix Ophiopogonis. However, fungal mycelia were clearly visible when air relative humidity, temperature, and moisture content were maintained at 75% RH, 25 °C, and 20%, respectively (**Figure 4** (**1b**, **2b**, and **3b**)). The mildew degree was greater when humidity, temperature, and moisture content were increased to 85% RH, 30 °C, and 30%, respectively (**Figure 4** (**1c**, **2c**, and **3c**)). Variance analysis results showed that the model equations were suitable for screening optimum storage conditions and predicting the occurrence of mildew. When the storage factors met the equations, they would be considered as proper storage conditions, but more extensive experiments were necessary to validate the applicability of the method in long-term storage. Further studies will be carried out to investigate the quality of Radix Ophiopogonis during storage to ensure the activity and safety of the natural product.

In this study, ergosterol was used as an indicator of fungal biomass to evaluate the degree of mildew. This not only quantified mildew degree but also extended the application of ergosterol assay to fungal invasion in agriculture products. Accompanied with the statistical method, this approach could reveal the relationship between storage factors and mildew degree. Furthermore, on the basis of the results, the methodology had great significance in finding suitable storage conditions for fungus-susceptible agriculture products; thus, more agricultural products could be prevented from developing mold by controlling the storage conditions. At the same time, this research mode might provide an effective and practical strategy to judge the degree and prognosis of the occurrence of fungus invasion on sugar-abundant agricultural products.

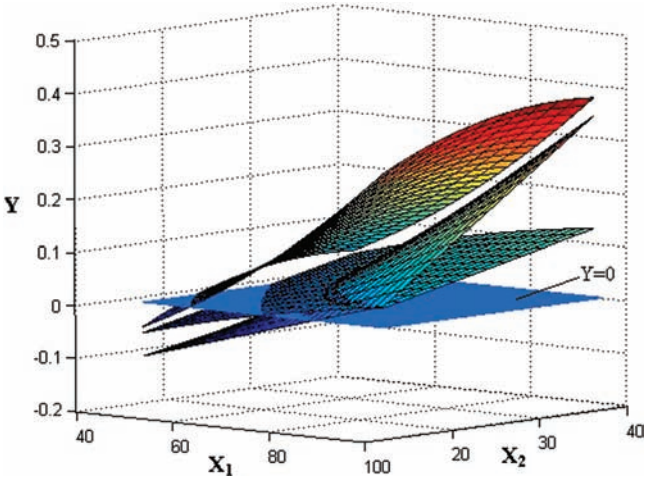

Figure 3. Three-dimensional response surfaces of the fungal biomass, air relative humidity, and temperature (moisture content constant at 18%).

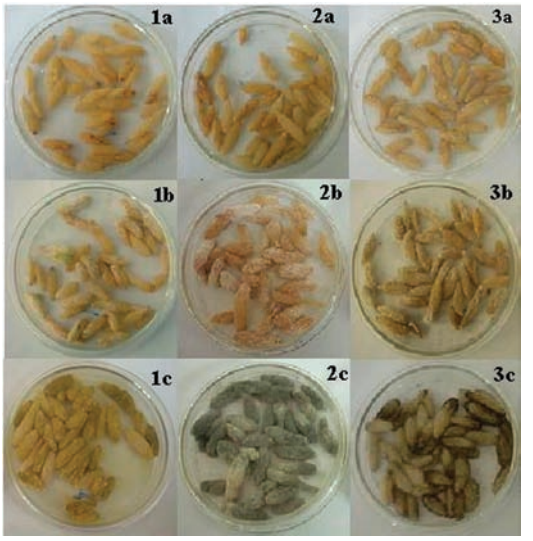

Figure 4. Degrees of mildew under different storage conditions: 1, Radix Ophiopogonis incubated with *A. flavus*; 2, Radix Ophiopogonis incubated with *G. avenacea*; 3, Radix Ophiopogonis incubated with *A. tubingensis*; Storage conditions: a, air relative humidity, temperature, and moisture content were 54% RH, 15 °C, and 18%, respectively; b, air relative humidity, temperature, and moisture content were 75% RH, 25 °C, and 20%, respectively; c, air relative humidity, temperature, and moisture content were 85% RH, 30 °C, and 30%, respectively.

Table 7. Results of Verification Experiments[a]

	factors			*A. flavus* (mg)		*G. avenacea* (mg)		*A. tubingensis* (mg)	
no.	X_1 (% RH)	X_2 (°C)	X_3 (%)	predicted value	exptl value	predicted value	exptl value	predicted value	exptl value
1	50	15	18						
2	54	15	18						
3	45	25	18					0.0218	0.0263
4	55	20	12					0.0412	0.0456
5	75	25	20	0.0811	0.0765	0.0595	0.0483	0.1939	0.1828
6	85	30	30	0.2442	0.2378	0.1628	0.1435	0.2879	0.2765
variance analysis			*F*	2.4		2.26		0.59	

[a] $F_{0.05} = 6.61$, $F < F_{0.05}$.

ACKNOWLEDGMENT

We acknowledge the technical support and assistance of Chuan-Chao Dai and Yan Chen from the Microbiology Department of the Nanjing Normal University. We thank Xiao-Ping Ding and Xiao-Qing Tang for critical reading and revising of the manuscript. We also gratefully acknowledge Chao Guo for his help on the statistical analysis of our data.

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Received for review December 17, 2009. Revised manuscript received March 23, 2010. Accepted April 2, 2010.