# Application of Exogenous Lipase in the Study of Rice Bran Stabilization

Mingyue Deng, Haixu Liu, Yongze Chai, Shaojin Wang, and Fang Gu*

***Abstract*—In view of the low content of lipase in rice bran, it is difficult to directly and efficiently measure its lipase activity in the rice bran heat inactivation experiment, an experimental method of adding exogenous lipase was proposed to quickly and accurately detect the lipase activity in the heated rice bran. Taking the correlation between the temperature and time of rice bran heat treatment and the enzyme activity as the starting point, the self-made aluminum heating container with high sealing property was used, and the COMSOL simulation verified that the container met the uniformity requirements of rice bran heating; Take rice bran as the heating medium, add a certain amount of exogenous plant lipase, and then heat the rice bran containing exogenous lipase, and detect the activity of lipase after heating, to explore the application of adding exogenous lipase in rice bran stabilization. The results showed that when exogenous lipase and rice bran were mixed with 1:1 (mass ratio), the lipase activity of the mixture increased in equal proportion; After heat treatment at 110 ℃, 120 ℃ and 130 ℃, the measured change of lipase activity in the mixture was in good agreement with the change trend of rice bran lipase activity, and the difference between different test temperature groups was significant (P<0.05), which provided an ideal detection model for the detection of lipase activity in the study of rice bran stabilization.**

***Index Terms*—Rice bran, lipase activity, stability, exogenous lipase**

## I. Introduction

Rice bran is a by-product of rice processing, consisting of pericarp, aleurone layer, sub-aleurone layer, seed coat, nucellus layer, embryo and endosperm [1]. The protein content of rice bran is about 14%–16%, the fat content is about 12%–23%, and the crude fiber content is about 8%–10% [2]; In addition, rice bran also contains calcium, manganese, iron, zinc and other mineral elements [3]. Rice bran oil extracted from rice bran contains three different natural antioxidants, tocopherol, tocotrienols and oryzanols [4], which have the ability to lower cholesterol, strengthen the immune system and control free radicals in the body, and are widely used in pharmaceutical, food and chemical industries [5, 6]. In 2019, China's rice output was 20.961 million tons, and the rice bran output exceeded 10 million tons. The weight of rice bran accounted for about 8%–10% of the weight of rice [2], which is very rich in resources. However, fresh rice bran is easy to be rancid, and the nutritional quality of the rancid rice bran and its products is reduced, resulting in its low utilization rate in industrial production. Therefore, the main way to maintain the quality of rice bran is to prevent rice bran from rancidity, which is the key factor to improve the utilization value of rice bran [7–9].

The main way to prevent rice bran rancidity is to reduce rice bran lipase activity and prevent lipases from catalyzing the hydrolysis of rice bran oil into free fatty acids, resulting in rapid rancidization of rice bran [10, 11]. Many expert scholars have done a lot of research works for rice bran stabilization and storage, and a variety of stabilization treatment methods have been adopted to reduce lipase activity in rice bran, to achieve the objective of improving rice bran storage behavior and stability. Such as reducing the lipase activity of rice bran by chemical synthesis, cold storage [6], boiling treatment [12, 13], extrusion [14], microwave treatment, etc. [15–19]. But because the amount of lipase in rice bran is very low and it is generally difficult to directly measure its activity in experiments, these stabilization treatments have all been used to determine whether rice bran lipase activity is reduced by a long-time storage experiment, in which researchers have to stably treat large amounts of rice bran for storage experiments [20], and to indirectly detect the inactivation rate of lipase by examining the change in fatty acid content during the storage period of rice bran. This largely limits the accuracy and timeliness of assay of rice bran lipase activity and cannot meet the demands of industrialized, batch produced rice bran, so rapid and accurate assay of rice bran lipase activity is important for rice bran stabilization research.

In this study, we used rice bran as heating medium and added a certain amount of exogenous plant lipase, then treated rice bran containing exogenous lipase by heating, and examined the activity of lipase after heating, and then investigated the application of adding exogenous lipase in the stabilization of rice bran. When exogenous lipase and rice bran were mixed 1:1 (mass ratio), a proportionate increase in mixture lipase activity was measured; Moreover, after heat treatment at 110 °C, 120 °C, and 130 °C, the measured variation pattern of lipase activity of the mixture corresponded well with the variation trend of rice bran lipase activity, and the differences among different tested temperature groups were significant ($P < 0.05$). This method can be used to rapidly and accurately detect the activity of lipase in rice bran after heating and can be used to prevent the rancidity and improve the storage behavior and stability of rice bran, providing some help for the future industrial and batch production of rice bran.

In view of the problem of low levels of intralipid lipase in rice bran, which cannot be detected by direct detection methods, this paper employed the addition of exogenous lipase to rice bran, and rapid detection of rice bran lipase activity upon inactivation could be achieved. In order to obtain the same effect when testing, the environment and

Manuscript received July 6, 2023; revised August 10, 2023; accepted Septeber 17, 2022.

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thermal conductivity of rice bran were simulated, exogenous lipase was added to rice bran for heating, and the experimental results showed that the measured change rule of lipase activity of the mixture corresponded well with the change trend of single rice bran lipase activity, and there was the same inactivation enzyme activity, which reached the purpose of simulating the environment of rice bran inactivation and increasing the detection efficiency of the enzyme inactivation in rice bran.

Aiming at the problems in the current research on rice bran stabilization, this study uses a self-made aluminum heating container with high sealing property, uses COMSOL simulation to verify the heating uniformity of the container, and heats the rice bran with some exogenous lipase, which improves the content of lipase in the rice bran in the micro environment, in order to quickly and accurately detect the change of lipase activity after heating, and provides a sensitive Easy-to-measure detection model.

## II. Materials and Methods

### A. Test Principle and Process

Due to the basic content of lipase in rice bran is low, the experimental effect is not obvious when the conventional method is used to directly determine the content of lipase in rice bran. Therefore, the mixture of exogenous lipase and rice bran can increase the content of lipase while simulating the material characteristics of rice bran, and Fig. 1 shows the test method of the research. By using the characteristic that lipase can catalyze the hydrolysis of oil ester to fatty acid, the activity of lipase can be calculated quickly and accurately by measuring the rate of fatty acid formation using copper soap method.detection method of the research experiment

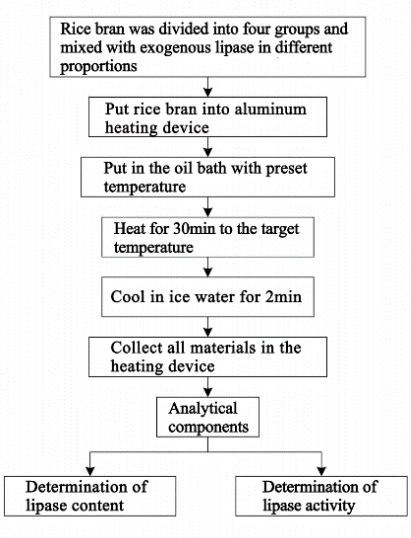



Fig. 1. Test method of experiment

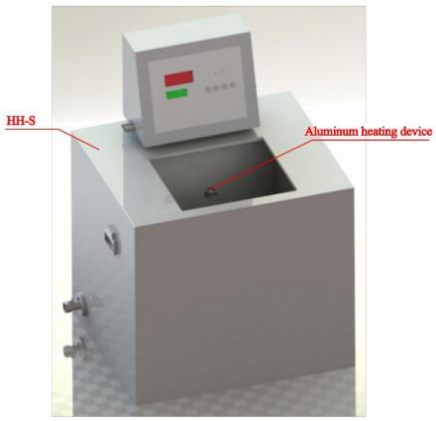



Fig. 2. Schematic diagram of heating device.

### B. Materials and Reagents

Fresh rice bran was purchased from Shaanxi Fujin rice Co., Ltd. rice bran was collected immediately after rice milling, passed through a 100 mesh sieve after crushing, filtered and placed in a freezer (FYL-YS-128L, Beijing Fuyi Electric Co., Ltd, Beijing, China) at −20 °C for storage and further use. The purchased commercial plant lipase (Hefei Bomei Biotechnology Co., Ltd, Hefei, Anhui), lipase content assay kit and lipase activity assay kit (Suzhou Keming Biotechnology Co., Ltd, Suzhou, Jiangsu, China) were stored at 4 °C for further use; Toluene 80 mL, ice and distilled water for use.

### C. Instruments and Equipment

A tabletop centrifuge (FRESCO 17, Thermo Co., Ltd, Massachusetts, America); Shaking homogenizer (Shanghai Fangrui Instrument Co., Ltd, Shanghai, China); Microplate reader (Synergy2, BioTek Co., Ltd, Vermont, America); Quartz 96 well plates (730.009-QG, Hellma Co., Ltd, Jena, Germany); Drying oven (SZF-6050, Shanghai Jinghong Experimental Equipment Co., Ltd, Shanghai, China); Water bath pan (Shanghai Medical Thermostat Equipment Factory, Shanghai, China); A CNC superthermostat (oil bath pan) (SC-30C, Ningbo Xinzhi Biotechnology Co., Ltd, Ningbo, China).

The container for rice bran heating is made by referring to the manufacturing method of aluminum heating elements designed by Washington State University [21], and optimized and improved. The schematic diagram of heating device is shown in Fig. 2 and its structure is shown in Fig. 3. The heating unit is composed of a base and a screw cap. This heating unit consists of two parts, a base and a screw cap, between which an O-shaped rubber ring provides good sealing properties to keep the sample moisture content constant; Its material has the characteristic of fast heat transfer, which enables the experimental material inside the heated vessel to warm up quickly and heat uniformly.

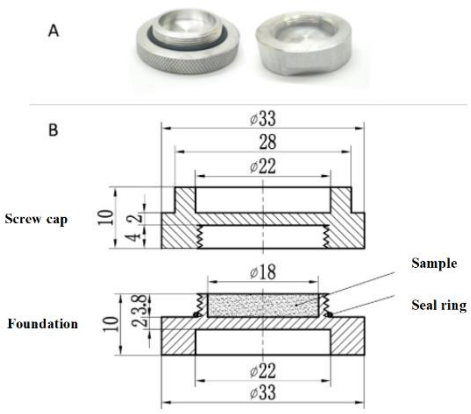



Fig. 3. Physical picutre (A) and schematic view (B) of aluminum test cells (Unit: mm).

### D. Experimental Methods

#### 1) Composition analysis of rice bran

Moisture content of rice bran before and after heat treatment was determined by constant weight method using GB 5009.3-2016 [22] 105 ℃; Crude protein determination was performed referring to GB 5009.5-2016 [23]; Crude fat determination was performed referring to GB 5009.6-2016 [24]; Fiber determination was carried out referring to GB 5009.88-2014 [25].

#### 2) Rice bran heating treatment

According to the report of Gong and Yu *et al.* [26], the temperature of the heat treatment target group is set at 110 ℃, 120 ℃, and 130 ℃, and the heating time is 30 min. The 25 ℃ group is heated for 30 min as the control group. Before heating to the target temperature, the lipase activity is detected at every 10 ℃ from 50 ℃ to monitor the change rule of lipase. In order to further verify the effect of heat treatment on the activity of lipase in rice bran, the experiment mixed exogenous lipase and rice bran at 0:1, 0.1:1, 0.5:1, and 1:1 respectively, and heat treated at 110 ℃, 120 ℃, and 130 ℃ in oil bath for 30 min, then detected the activity of lipase.

For heat treatment, 1 g fresh rice bran was placed inside the homemade aluminum heating device, the material was spread out evenly, the cover was tightened, placed in the preset good temperature oil bath pot after heating for 30 min, quickly placed in ice water to cool for 2 min, and collected the whole material inside the heating device for subsequent tests.

#### 3) Lipase content determination in rice bran

ELISA method was used to detect the lipase content in rice bran, the kit coated microplates with purified antibodies against plant lipase to make solid-phase antibodies, added lipase to the micro wells coated with single antibodies in turn, and bound with horseradish peroxidase labeled lipase antibodies to form antibody antigen enzyme labeled antibody complexes, after thorough washing and substrate color development, the dark color and the lipase content in the samples were positively correlated. The absorbance was determined with a microplate reader at a wavelength of 450 nm, and the concentration of plant lipase in the samples was calculated by a standard curve. Before the start of the test, 1 g of rice bran was suspended in phosphate buffered saline and placed on ice to be homogenized by a mortar, and the subsequent procedures were performed strictly according to the instructions.

#### 4) Measurement of Lipase (LPS) activity in rice bran

The rate of lipase catalyzed hydrolysis of oleyl esters to fatty acids before and after heat treatment was measured using the copper soap method, and the lipase activity was calculated. The unit of lipase activity is defined as 1 μmol of fatty acid produced by hydrolyzing olive oil per gram of rice bran per minute at 37 ℃.

Configuration of liquids required for assay: reagent one, liquids 65 mL × 2 vials, stored at 4 °C; Reagent II, 10 mL liquid × 1 vial, store at 4 °C (shake vigorously for 20 minutes with a shaking homogenizer before each use); Reagent three, 80 mL toluene × 1 vial, stored at 4 °C; Reagent IV, liquid 10 mL × 1 vial, stored at 4 °C; Standard, liquid 10.0 μ L × 1 vial, 10 μ mol / mL standard solution) and stored at 4 °C. Add 3.168 mL toluene before clinical use, dissolve well.

For the lipase assay, the enzyme preheated the microplate reader for 30 minutes, adjusted the wavelength to 710 nm, and adjusted the distilled water to zero. Reagent 1 and 2 were placed in a 37 ℃ water bath for preheat for 30minutes. Then two 1.5 mL EP tubes were taken as blank tubes and measuring tubes respectively. 150 μ L of distilled water and 300 μ L of reagent were added to the blank tube. 50 μ L of samples, 300 μ L of reagent 1 and 100 μ L of reagent 2 were added to the assay tube. Both the blank tubes and the measurement tubes were placed in the 37 ℃ oscillation reaction for 10 minutes. After the oscillation, 800 μ L was added to the blank tube and the measurement tube.8000g was centrifuged at 10 minutes after shaking at 25 ℃ for 10minutes, and the supernatant was removed. Another three 1.5 mL EP tubes were taken as blank tubes, measuring tubes and standard tubes. Add 400 μ L of supernatant and 100 μ L of reagent to the blank and test tubes. Add 400 μ L of standard and 100 μ L of reagent to the standard tube. After 5 minutes of 37 ℃ shaking, the were left for 5 minutes, and 200 μ L of superfluid was added to trace quartz 96-well plates, and the absorb value was measured at 710 nm. Lipase activity in rice bran can be obtained.

$$\begin{aligned} LPS\ (\mu mol/min/g) &= [\text{standard C} \times (\text{testing tube A} - \text{blank tube A}) \\ &\div (\text{standard tube A} - \text{blank tube A})] \times \text{total V} \\ &\div (\text{standard W} \times \text{V} \div \text{total number of sample V}) \div \text{T} \end{aligned} \quad (1)$$

In the above formula, standard C is 10 μ mol/mL; Total V reaction is the total reaction volume, 0.8 mL; V sample is the volume of sample added in the reaction, 0.05 mL; The total volume of V sample is 1 mL.

#### 5) Statistical analysis

All the tests were performed in triplicate, and the test data were expressed as mean ± standard deviation. The coefficient of determination R2 was used to assess the degree of model fit, and the data were statistically analyzed at a 1% or 5% significance level with SPSS 17.0 statistical software.

## III. Results and Analysis

### A. Analysis of Basic Components of Rice Bran

The results showed that the water, crude protein, fat and fiber components of fresh rice bran without heat treatment were close to domestic literature; the content in rice bran did

not change significantly before and after 110 ℃ treatment (Table I). The water content in rice bran plays an important role in the activity of lipase and its acidification in rice bran [27], This test was heat-treated at 110 ℃, which is already higher than the evaporation temperature of the water. Before and after heat treatment, the moisture content in the rice bran did not change significantly, indicating that the self-made aluminum heating device used in the test could protect the constant moisture of the material, so as to avoid the influence of lipase activity detection data due to water loss in the subsequent test.

TABLE I: BASIC COMPOSITION ANALYSIS OF RICE BRAN

| Group | Water content /% | Crude protein /% | Fat /% | Fiber /% |
|---|---|---|---|---|
| Before heat treatment | 12.6±0.13 | 13.4±0.39 | 20.1±1.12 | 9.23±0.14 |
| After heat treatment | 11.2±0.21 | 14.4±0.61 | 18.9±2.14 | 8.96±0.37 |

### *B. Simulation of Heat Conduction in Heating Vessel Based on COMSOL*

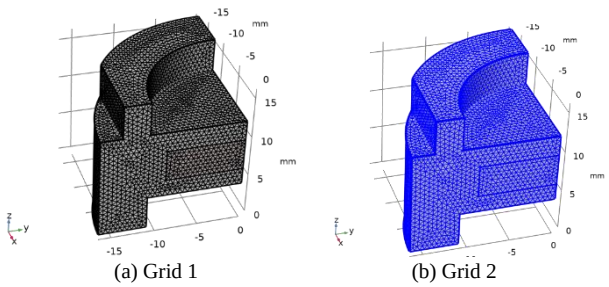
#### *1) Construction of 3D model of heating vessel*

The SolidWorks software was applied to model the aluminum heating device, and the generated model was imported into COMSOL for thermal conduction simulation to verify that the heating vessel contents were heated uniformly and stable, which could meet the experimental requirements.

#### *2) Grid division of heated containers*

Aluminum with thermal conductivity of 27W / (m·K) is used as the matrix material. In the model, the thermal conductivity of the rice bran material is 0.2 W / (m·K), and the rest is the air gap. The defined virtual simulation model sizes are the same as the actual model size.

The resulting models were meshed according to different curvature factors, narrow area resolution and maximum cell growth rate. Where grid 1 has a maximum cell size of 1.65 mm, a minimum cell of 0.297 mm, a maximum cell growth rate of 1.5 and a curvature factor of 0.6. Grid 2 has a maximum cell size of 0.578 mm, a minimum cell size of 0.0248 mm, a maximum cell growth rate of 1.35 and a curvature factor of 0.3. The grid division is shown in Fig. 4.



(a) Grid 1 (b) Grid 2

Fig. 4. Schematic diagram of grid division.

#### *3) Heating simulation and results*

Heating simulation analysis of aluminum heating container by COMSOL, the calculation formula of heat conduction is:

$$\rho C_p \frac{\partial_T}{\partial t} + \rho C_p u \cdot \nabla T + \nabla \cdot q = Q + Q_{ted}$$
$$q = -k\nabla T \quad (2)$$

$\rho$ is the material density; $q$ is the heat flux; $C_p$ is the constant pressure of the material $\frac{\partial_T}{\partial t}$ is the bias of temperature to time; $\nabla T$ is the temperature gradient; $Q$ is the heat; $Q_{ted}$ is the heat source; $k$ is the thermal conductivity; $\nabla$ is the Hamiltonian operator; $q$ is the temperature rate of change; $u$ is the temperature coefficient.

The simulation results are shown in Fig. 5, indicating that after 30 min of 110 ℃ heat treatment, the rice bran in the aluminum heating device can be heated evenly, and the highest temperature can reach the expected set temperature of 110 ℃, which ensures that the contents of the container can be heated evenly and stable and meet the experimental requirements.

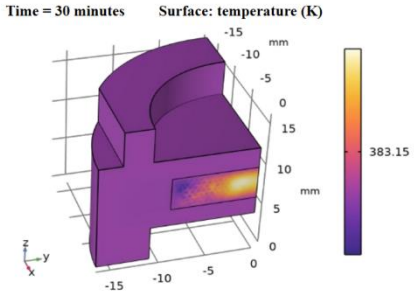
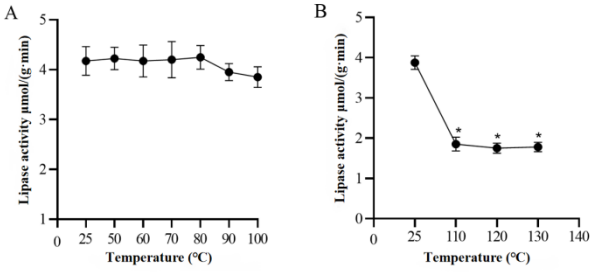



Fig. 5. Simulation results of Container temperature distribution diagram.

### *C. Effect of Temperature on the Lipase Activity of Fresh Rice Bran*

It was found that the basal content of lipase in the fresh rice bran was (6.70±0.27) ng / g and the lipase activity was (4.18±0.29) μ mol / (g. min). At room temperature (25 ℃) as the control group, heating for 30 minutes from 60 ℃–100 ℃ had no significant effect on lipase activity in Fig. 6 (a), lipase activity decreased with the temperature from 80 ℃–100 ℃, but no significant difference was found by statistical analysis ($P > 0.05$). This indicates that rice bran at about 12% constant water and heated within 100 ℃ for 30 minutes showed no significant inhibition of the lipase activity in rice bran. However, heating at 110 ℃ for 30 minutes significantly reduced the lipase activity in the rice bran, which was 44.3% of the lipase activity of fresh rice bran. However, with the heating temperature increasing to 120 ℃ and 130 ℃, no further improvement of lipase inactivation was detected ($P > 0.05$) in Fig. 6 (b), it is speculated that due to the relatively low basal content of lipase in rice bran, it is not enough to detect the change of lipase activity under the existing test conditions.



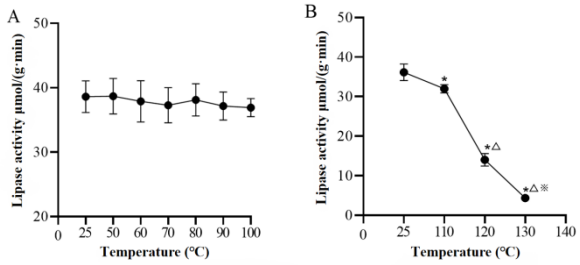

A. Effect on rice bran lipase activity during heating in the range of 60 ℃–100 ℃; B. Effect on rice bran lipase activity when heated in the range of 110 ℃–130 ℃.

* $P$ <0.05 as compared to the controls

Fig. 6. Effects of temperature on lipase activity of rice bran.

### D. Effect of Temperature on Commercial Plant Lipase Activity

The result shows that, Compared to the 25 ℃ control group [(36.13 ± 2.10) μ mol / (g · min)], Heating for 30 minutes in the 60 ℃–100 ℃ range, Also had no effect on commercial lipase activity (Fig. 7A, $P > 0.05$), However, in the range of 110 ℃–130 ℃, With the temperature, Stepwise reduction in lipase activity (Fig.7B), At the time of 110 ℃, After heating for 30 min at 120 ℃ and 130 ℃, The lipase activity fraction was successively (31.93±1.07) (14.03±1.57) (4.35±0.60) μ mol / (g min), significant differences were found between the groups ($P < 0.05$).




A. Effect on commercial plant lipase activity during heating in 60 ℃–100 ℃; B. Effect on commercial plant lipase activity during heating in the 110 ℃–130 ℃ range.
Compared with the control group, * $P < 0.05$; compared with 110 ℃, $^{\triangle}P < 0.05$; compared to 120 ℃, $^{※}P < 0.05$
Fig. 7. Effects of temperature on comercial pure lipase activity.

### E. Effect of Temperature on Lipase Activity in Rice Bran after Addition of Exogenous Lipase

Due to the low basal content of lipase in rice bran, the test further investigated whether the obvious inactivation difference could be obtained by adding exogenous lipase to rice bran. The lipase activity in rice bran was measured at 0:1, 0.1:1, 0.5:1:1 and 1:1. It was found that the lipase activity increased lipase activity in rice bran (Fig. 8). According to the above results, at room temperature, 1:1 mixed exogenous activity, the mixture lipase activity is about 9 times that of lipase activity in raw rice bran (38.6/4.2), indicating that the addition of exogenous lipase in a 1:1 ratio in rice bran can significantly improve the sensitivity of rice bran lipase activity detection. Therefore, a 1:1 mixture of exogenous, lipase and rice bran, treated with 110 ℃, 120 ℃, and 130 ℃ of the mixture was shown in Table II.

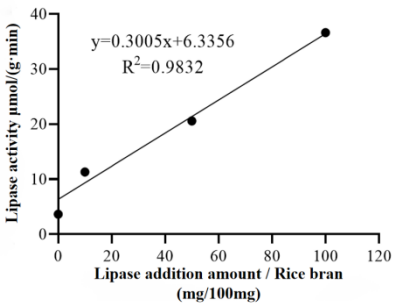



Fig. 8. Changes of lipase activity of rice bran after supplemnt of exogenous lipase.

The results found that with the increase of temperature, the lipase activity gradually decreased. When the temperature reached 130 ℃, the lipase inactivation rate of lipase was close to 90%, meeting the requirements of rice bran stabilization treatment, which was in good agreement with the results of lipase inactivation test in previous literature. Comparisons between the different test temperature groups showed significant differences ($P < 0.05$) (Table II).

TABLE II: EFFECTS OF TEMPERATURE ON LIPASE ACTIVITY OF RICE BRAN AFTER SUPPLEMENT OF EXOGENOUS LIPASE

| Temperature (℃) | Lipase activity (μ mol / min) | Lipase inactivation rate of (%) |
|---|---|---|
| 25 | 36.63±3.42 | — |
| 110 | 30.93±4.46* | 15.6 |
| 120 | 14.27±1.91*△ | 60.9 |
| 130 | 4.02±0.05*△▲ | 89.1 |

Note: 1) Compared with the control group, * $P$ <0.01; compared with 110 ℃, $^{\triangle}P$ <0.01; compared to the comparison with 120 ℃, $^{\blacktriangle}P$ <0.01.

## IV. CONCLUSION

1) When fresh rice bran was heated at 110 ℃ for 30 minutes, the lipase activity of lipase in rice bran was significantly reduced, which was 44.3% of that of fresh rice bran; however, as the heating temperature increased to 120 ℃ and 130 ℃, no further improvement of lipase inactivation was detected, indicating that the lipase content of lipase in rice bran is low and difficult to detect.

2) After the commercial plant lipase was heat treated at 110 ℃, 120 ℃ and 130 ℃ for 30 min, its activity gradually decreased with the increase of temperature, showing temperature dependence, and the comparison difference between groups was significant ($P$ <0.05).

3) When pure lipase was added to rice bran at 1:1 (mass ratio), the lipase activity of the mixture increased in equal proportion; After heat treatment at 110 ℃, 120 ℃ and 130 ℃, the change of lipase activity showed the same temperature dependence as that of pure lipase, and there was significant difference between different test temperature groups ($P < 0.05$). When the temperature reaches 130 ℃, the lipase inactivation rate is close to 90%, which meets the requirements of rice bran stabilization treatment, and is in good agreement with the lipase inactivation test results described in the previous literature.

4) It can be seen from the test results that the addition of exogenous lipase in rice bran can significantly improve the sensitivity of rice bran lipase to the effect of temperature, and the test results can reflect the effect of rice bran heating to kill the enzyme, without long-term storage experiment. This greatly improves the convenience and accuracy of lipase activity detection in rice bran, improves the feasibility of lipase activity detection in rice bran at high temperature (120 ℃ - 130 ℃), and provides an ideal detection model for rice bran stabilization research.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Fang Gu and Shaojin Wang conducted the research, Mingyue Deng, Haixu Liu and Yongze Chai analyzed the data and conducted the model simulation, and Mingyue Deng wrote the paper. All authors had approved the final version.

## FUNDING

This research has received support from the Shaanxi Key Research and Development Program of China (2019NY-171, 2019ZDLNY02-04), National Natural Science Foundation of China (31971805), National Key Research and Development Program of China (2019YFD1002401), And Innovative Training Program for College Students of Northwest A&F University (No. S202010712178, S202010712700). The authors are also gratefully to the reviewers for their helpful comments and recommendations, which make the presentation better.

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