

## RESEARCH ARTICLE

# Quality formation of germination brown rice under microwave drying: from GABA content to color value

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## ABSTRACT

In order to improve the final quality of germination brown rice (GBR), the changes of GBR in Gamma-aminobutyric acid (GABA) and color are investigated under microwave drying conditions. Results indicate that Microwave drying process may improve GABA in GBR due to microwave volumetric heating inducing the glutamic acid decarboxylase (GAD) activity; and the dried GBR with golden appearance under the microwave intensity of 2.75 W/g and the apparent velocity of 2.50 m/s considering the drying efficiency and quality of dried GBR. In this study, microwave drying conditions not only improve the drying efficiency of GBR, but also improve the appearance and color of GBR after drying, reduce GABA degradation and control the generation of burst rate.

**Keywords:**  $\gamma$ -aminobutyric acid (GABA); color; germination brown rice (GBR); microwave drying; quality

## INTRODUCTION

Germination brown rice (GBR), as new wholegrains product, is rich in nutritional ingredients, such as  $\gamma$ -aminobutyric acid (GABA), reducing sugar (monosaccharide) and amino acid, which has higher nutrition and health value than that of brown rice and polished rice (Zhang et al., 2019). GBR is produced as brown rice immersed in clear water in temperature of 35-45 °C for 24h to sprout 1.5-2.0mm buds (Shen, 2019). In the process of germination, the enzymes in brown rice including amylase, lipase, peroxidase and catalase are activated and released, which causes the enzymatic hydrolysis reaction between starch and protein to generate small molecules substance (Wunthunarat et al., 2019). GBR has been processed into characteristic foods, such as bread (Wunthunarat et al., 2020), rice flour (Qi et al., 2019) consisting rich GABA, to express attractive development potential as health food of wholegrains (Ng et al., 2013). How to keep the stabilization of nutritional ingredients in GBR in further processing is valuable research topic (Cornejo et al., 2015), which has been highly concerned by food experts and consumers (Ti et al., 2014; Li et al., 2018).

However, fresh GBR is easily suffered from microbial invasion due to moisture content higher than 30% (w.b),

leading to the quality spoilage and the loss of nutrition and commodity value. The rapid and timely drying of GBR to the moisture content of 13.0%~14.5% (w.b) is not only beneficial to extend safe storage, but also provide quality guarantee for the further processing of follow-up products. Research efforts of GBR drying has been focused on the optimization of capability, efficiency of dryer and quality control of final product (Huang et al., 2012).

Microwave drying has remarkable advantages of high drying rate, great energy efficiency, and easy controllability, which can meet the demand of fresh GBR (Shen, 2020). The optimization of the microwave power and drying duration improves the contents of reducing sugars, free amino acid, soluble protein and ascorbic acid in the GBR dried, and reduces the gelatinization temperature (Sun et al., 2016) to enhance the quality of dried GBR with low hardness and high content of GABA under relative humidity of 72.18%, drying temperature of 37.80 °C, drying duration of 10.37 h (Han, 2013). Existing research results proved that the efficiency of microwave drying GBR is significantly higher than that of routine hot air drying (Zheng et al., 2015; Ito and Ishikawa, 2014). However, the complex mechanisms of drying conditions interacting GBR results in variable quality of dried GBR, especially in

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GABA content and appearance color (Yu, 2016; Zhang, 2016). At current research, the change of GABA content in GBR with the drying temperature is confused that GABA was not sensitive to drying temperature of 90-150°C (Chungcharoen et al., 2015), conversely, the GABA content in GBR had negative relation with temperature in the range of 50-75 °C under hot air drying for GBR (Yang et al., 2013). Shen found (2020) that increasing microwave intensity accelerates the color changes of GBR, and the critical temperature to avoid serious browning and charring kernels inside material layer was 132 °C and 170 °C, respectively, and the GABA content of GBR overall tended to decrease, and suitable average temperature to retain high GABA content may be governed in the range of 64~67 °C, which can provide parameter basis for retaining high GABA content of GBR (Shen et al., 2021). The suitable technology parameters to achieve final quality for continuous microwave drying of GBR were determined as microwave intensity of 4 W/g, air velocity of 1.0 m/s, drying time of 10 min for per drying pass and tempering ratio of 1:2 (Shen et al., 2020). Existing researches on the quality of GBR mainly are focused on the effects of microwave drying parameters on the changes of GABA and color. Little investigation is done about kinetic analysis of changes of GABA and color of GBR under microwave drying, which limits the extension of optimal microwave drying technology and quality control for GBR.

Based on above-mentioned, research objectives are as follows: 1) investigation of the kinetic characteristics of quality formation in terms of GABA content and color of germination brown rice under microwave drying; 2) optimization of microwave drying conditions considering drying quality of final GBR. The control proposal of drying quality of GBR provides technical basis for drying production of high-quality GBR.

## MATERIALS AND METHODS

### Raw material

Fresh active rice was provided by Heilongjiang Jindu Rice Industry Co., Ltd. Brown rice germinated under standard production conditions (germination temperature 35-37 °C, relative humidity of germination environment 95% - 98%), was naturally cooled to room temperature (18-20 °C), and then was transported to the agricultural products processing laboratory of Engineering College of Northeast Agricultural University, was sealed at low temperature for subsequent microwave drying experiment.

### Measurement method

Taking the refrigerated sealed bag of GBR and put it in the laboratory for 4-6 h at the room temperature

(20-22 °C), and the initial moisture content is 28.8% (*m.b*) for the microwave drying experiment (as shown Fig.1(a)). The microwave drying experiment was carried out in a continuous microwave dryer. The dried GBR was naturally cooled. 2000g GBR sample was weighed and sealed in a plastic bag to measure the quality index.

### Moisture content of GBR

The moisture content of GBR samples were measured by using oven method in 105°C about 3-4 h to constant weight for each sample of 5g.

### Temperature measurement

The infrared thermometer (gm1150, Dongguan Zhixuan Instrument Co., Ltd., Dongguan, China) is used to measure the temperature for each GBR sample.

### Color measurement

The color of GBR was measured by a colorimeter (CR-20, Konica Minolta, Inc., Japan). The color scale was expressed in  $L^*$ ,  $a^*$ , and  $b^*$  by the CIELAB color space.  $L^*$  indicates brightness or darkness in the range of 0-100, and  $a^*$  represents redness (+) and greenness (-), and  $b^*$  denotes yellowness (+) and blueness (-). The color value for each sample was measured for eight replicates. In addition, the total color difference ( $\Delta E$ ) and the chroma ( $C^*$ ) were derived from the measured color values according to Eqs. (1)-(2) (Shen et al., 2021):

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (1)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (2)$$

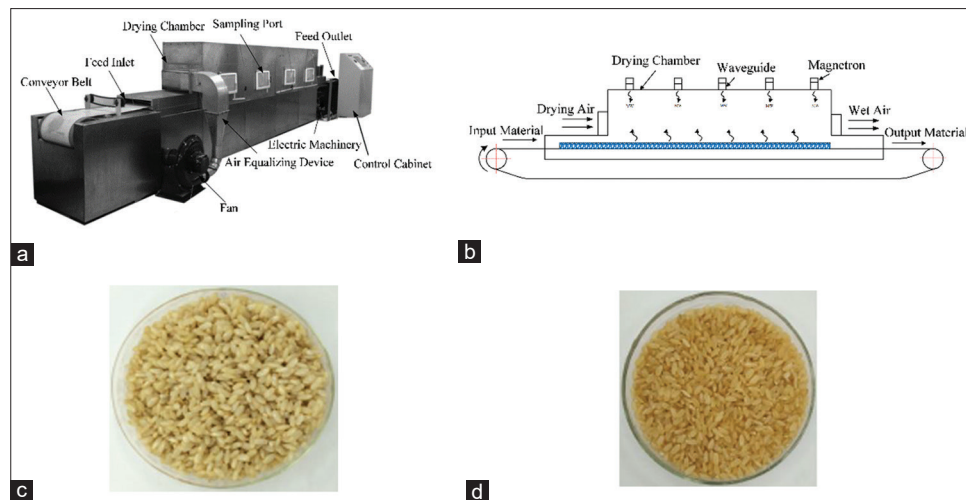
where  $L_0^*$ ,  $a_0^*$  and  $b_0^*$  are the color values of GBR in shade sun drying;  $L^*$ ,  $a^*$  and  $b^*$  are the color values of GBR in microwave drying.

### GABA determination

Liquid chromatography is employed to determine the GABA content in GBR. HPLC measure conditions as A: B at 72:28; mobile phase A: sodium acetate of 20 mmol/L pH 6.34; mobile phase B: methanol 100%; flow velocity of 0.8 mL/min; injection volume 20 mg. Detection wavelength of 338 nm; column temperature of 40 °C, the relative deviation of this method is 1.06%, the detection limit is in 5 mg/100g, and the recovery rate is in range of 93.2%-105%.

### GAD determination

GAD activity was detected by colorimetry and determined by GAD detection kit (Suzhou Keming Biotechnology Co., Ltd.). In short, tissue samples were prepared



**Fig 1.** Microwave dryer (a) schematic diagram (b), and fresh GBR(c) and dried GBR (d).

according to the ratio of tissue mass (g) to extraction volume (ML) 1:10, and then homogenized in an ice bath. The samples were prepared at 8000 rpm at 4 ° C × G. centrifuge at the rotating speed for 10 min, extract the supernatant and place it on ice for subsequent test. Mix the supernatant with phosphate buffer (200 μ 50. 20 mmol/L, pH 7.2), and the mixture was reacted in a water bath at 40 °C for 1h, and then put into an ice bath. Subsequently, the reaction solution was mixed with 200 μ L borate buffer (0.2 mol/L, pH 9.0), 100 μ L re evaporate phenol solution (6%) and 400 μ L sodium hypochlorite (10%) mixed. After sufficient shaking, hold the mixture in a 95 ° C water bath for 10 minutes, then place it in an ice bath for 20 minutes and continue shaking until a blue-green compound appears. After preparation, 200 μ L of the reaction solution was placed in a 96 well microplate, then placed in a microplate reader and shaken for 10 s to ensure complete mixing. Monitor the change of absorbance value at 640 nm at 40 ° C. Under standard conditions, calculate the absorbance of the sample to be tested by:  $A = 0.0682c - 0.0432$  ( $R^2=0.999$ ) (Where C is the concentration of the standard sample (μ mol/mL); A is the absorbance of the sample to be tested (μ mol/mL). GAD activity was defined as 1 per mg tissue protein per minute μ Mol GABA catalytic product and calculated according to the absorbance obtained and the protein concentration of the sample as  $Q = 0.733 [(A_m - A_c) + 0.0432] / c_{pr}$  (Where Q is the activity of GAD (μ mol/min/mg prot);  $A_m$  is the absorbance of the sample to be tested (μ mol/mL);  $A_c$  is the absorbance of the reference substance (μ mol/mL);  $c_{pr}$  is the sample protein concentration (mg-prot/mL) determined by using the protein quantitative detection kit.

### Experimental design

In order to analyze the effects of microwave drying conditions on the quality of GBR, to evaluate and control

the quality of GBR dried, on the basis of the research results of microwave drying characteristics of GBR (Sun, 2016), microwave intensity, air-flow velocity and drying duration per cycle were selected as influencing factors, GABA, protein and color of GBR were selected as quality evaluation index. Factor level coding is shown in Table 1.

### Data processing

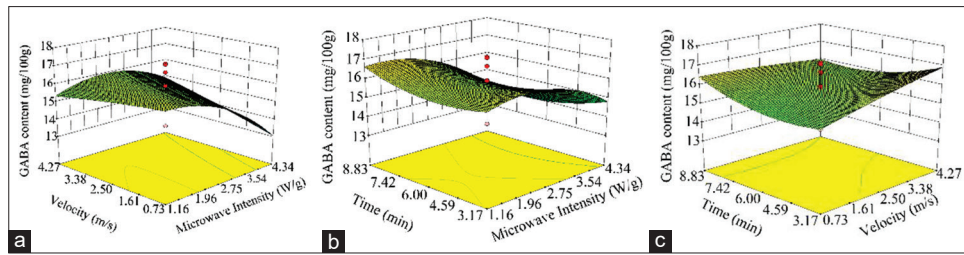
All experimental data measured three times was presented as the mean values ± standard deviation. Statistical significance was evaluated by analysis of variance (ANOVA) and Duncan's multiple range test at 95% significance level ( $P<0.05$ ) using SPSS software (Ver. 22.0, SPSS Inc., Chicago, USA). Finding the relationship between the experimental factors and quality indexes by means of Sigmaplot software (Ver 12.5, SYSTAT Inc., US). Design Expert software (Ver 10.0, Stat-Ease, Inc.) was employed to analyze statically the experimental data from Table 1 and plot the response curve surfaces.

## RESULTS AND DISCUSSION

### The change kinetic of GABA in GBA under microwave drying conditions

γ- aminobutyric acid (GABA), as a characteristic component of GBR, determines final quality of dried GBR. Therefore, the analysis of the influence of microwave drying conditions on GABA content is the basis of evaluating and controlling final quality of dried GBR. Microwave intensity had the most significant effect on the content of GABA in GBR, followed by drying time and apparent air-flow velocity (Sun, 2016). The effects of microwave drying conditions on GABA content in GBR is shown in Fig. 2.

$$\gamma = \gamma_0 \exp(-kt) \quad (3)$$



**Fig 2.** Effects of microwave drying conditions on GABA content in GBR Based on results from Fig. 2 (a), (b) and (c), the content of GABA in GBR decreased with the increase of microwave intensity during drying. However, the air-flow velocity and drying duration had no significant effect in statically on the content of GABA in GBR. The first-order kinetic can be used to describe the decrease of GABA content in GBR during microwave drying as shown in Eqn. (3) (Peron, 2017).

where,  $k$ - Reaction rate constant, ( $\text{min}^{-1}$ ),  $\gamma$ - Active substance content at time  $t$ (mg),  $t$ - Drying time(min),  $\gamma_0$  - Initial component content (mg).

In the conditions of microwave drying, the temperature of GBR increase at constant microwave output power. The ratio of mass to power ( $m/p$ ) instead of temperature was introduced into the improved Arrhenius equation as shown in Eqn.(4), which characterizes the influence of microwave intensity on dynamic parameters. In the process of microwave drying, the  $m/p$  value decreases with the increase of microwave intensity  $MI$  at constant microwave input power  $p$ . The  $m/p$  value of material decreases due to the decrease of water evaporation mass. According to the formula  $k=A\exp(-E^*m/p)$ , the higher  $m/p$  leads to the greater  $k$  to indicate the more obvious degree of GABA degradation.

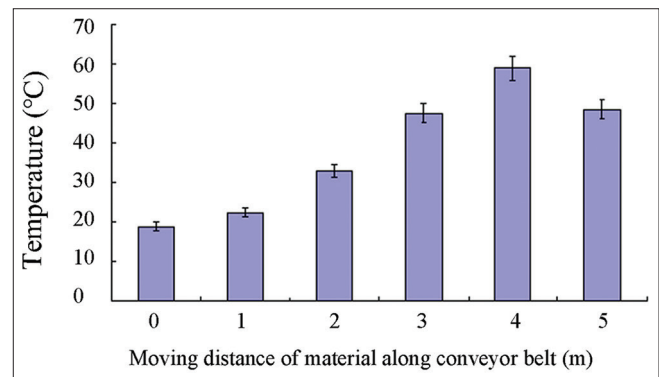
Arrhenius equation characterizes the change of reaction rate constant with temperature as shown in Eqn.(4).

$$k = A \exp(-E_0 / RT) \quad (4)$$

where,  $T$ -Absolute temperature, K,  $E_0$ - Activation energy of reaction,  $\text{kJ} \cdot (\text{mol} \cdot \text{k})^{-1}$ ,  $R$  - Gas constant is  $8.314 \text{ J} \cdot (\text{mol} \cdot \text{k})^{-1}$ ,  $A$  - pre-factor.

As shown in Fig.3, the temperature of GBR material layer tends to the increasing trend along the moving direction of conveying belt in microwave drying of GBR, where the higher temperature may lead to the more degradation of GABA.

The experiment presented in Fig. 4 was carried out on a continuous microwave dryer with a four-pass drying process. Five sampling points are uniformly selected along the movement direction of the conveyor belt on the dryer as shown in Fig.1. After the microwave drying process of GBR reaches a stable state, the sample of GBR is taken out from the sampling port, and the temperature, moisture content and GABA content are measured, as shown in Fig.4 and Fig.5.



**Fig 3.** Temperature distribution of microwave drying activity rice along conveying direction.

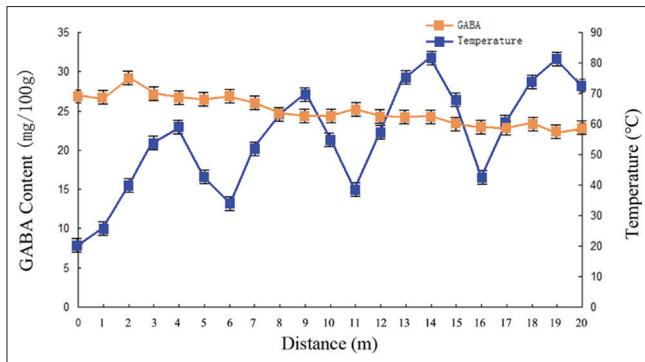
According to the changing trends of GABA content of GBR in the microwave drying process in Fig.4, Exponent Decay Function with four-parameter in the Sigmaplot software library function is selected as  $f=a*\exp(-b*x)+c*\exp(-d*x)$  to fit the GABA content data. According to data shown in Fig.4, the model of GABA content describing the changes of GBR during microwave drying process was fitted as Eqn.(5):

$$\frac{C}{C_0} = 0.0737 \exp(-1.0295t) + 0.9138 \exp(-0.0052t) \quad (5)$$

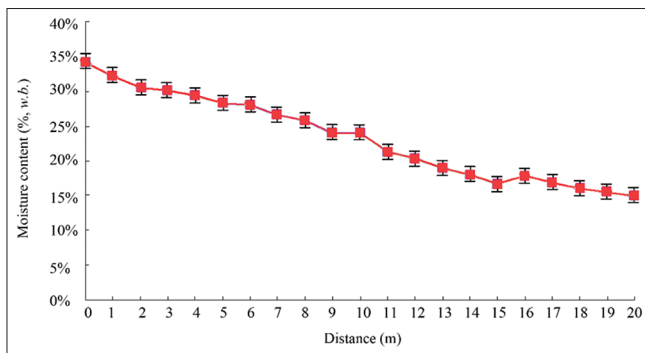
(Determination coefficient  $R^2=0.8554$ , and standard deviation  $SEE=0.0176$ ,  $p<0.05$ )

Fitting model (5) may reflect the change stages of GABA content in GBA under microwave drying, which provides reliable trends in good agreement with the practical case (Sun, 2016), although its determination coefficient  $R^2$  of 0.8554 less than 0.9. According to Eqn. (5), in the second drying pass, GABA degradation power is higher than that in the third pass. As shown in Fig.6, GAD activity in GBR firstly increase with microwave drying, the GBR appropriate temperature in  $33.93\text{--}69.71^\circ\text{C}$  is suitable for the excitation of GAD activity and moisture content was at a high level (27.95%–24.00%), which was conducive to the movement of active molecules and promotes synthesis of GABA. In the third drying pass, the temperature increased from





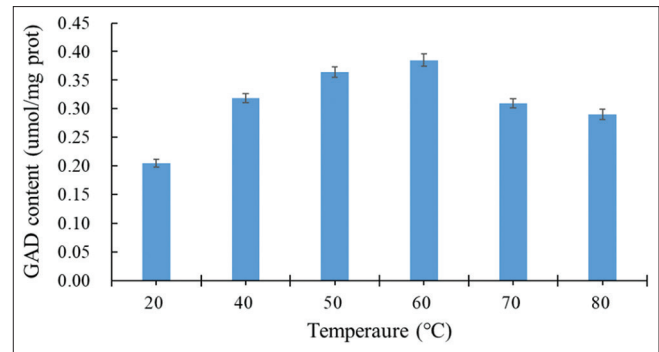
**Fig 4.** Changes of GABA content and temperature of GBR during microwave drying.



**Fig 5.** Change of moisture content in microwave drying process.

41.98 to 81.36 °C, but the moisture content decreased from 17.89% to 14.92%. The reduction of moisture content was not conducive to GABA synthesis (Shen et al., 2021).

The principle of the enrichment of GABA in brown rice is attributed to activation of endogenous Glutamate decarboxylase (GAD) and conversion of the free glutamate in brown rice into GABA with obvious increase of internal reducing sugars and free amino acids (including glutamate Glu). The optimum reaction temperature range of GAD was 25-40 °C for GBR. As shown in Fig.6, GAD activity increase with temperature of GBR. The high activity of GAD promotes the synthesis and enrichment of GABA (Wang, 2012). The liquid chromatogram of GABA in raw GBR and the sample dried by microwave at each pass (a total of four passes) are shown in Fig.7. The GBR dried pass through the microwave dryer in 96s from the inlet to reach the second sampling port (#1-#2), as shown in Fig.4. When the temperature of the GBR reaches 39.78°C and the moisture content of 30.50%, the activity of glutamate decarboxylase GAD in brown rice promotes the synthesis of GABA from free Glu and proteolytic Glu in the GBR at the following drying pass. Therefore, GABA content in the GBR increases at initial stage of microwave drying as shown in Fig.4. With the alternation of microwave drying and tempering process, the temperature of GBR



**Fig 6.** Change of GAD activity in GBR with microwave drying.

increased in a fluctuating manner as shown in Fig.4, which causes the increase of GAD activity in GBR and promoted the production of GABA (Gu and Jiang, 2002). However, GABA synthesis may be hindered, and GABA increment is limited due to the decrease of moisture content in GBR. With the increase of temperature, the GAD activity in GBR decreases (as shown in Fig.6), and the substrate Glu concentration decreases or the coenzyme concentration is insufficient, which weakens the GABA synthesis ability in GBR. Under microwave drying conditions, pyruvate transaminase in GBR can catalyze GABA to produce succinic semialdehyde, resulting in the decrease of GABA content. The comprehensive effect of the above-mentioned process results in the lower GABA production in GBR than that of consumption. During the second pass and the third pass of microwave dryer, no significant change of GABA content in GBR was found in views of statistics.

According to above-mentioned results, the feasible temperature (33.93-69.71oC) of GBR during microwave drying can stimulate GAD activity and higher moisture content (27.95%→24.00%), which was beneficial to the motion of active molecules and the synthesis of GABA. In the drying stage with the temperature rise from 41.98 to 81.36°C and the moisture content decrease from 17.89% to 14.92%, GAD is inactivated at high temperature and low moisture content impede GABA synthesis as shown in Fig.6. In the initial stage of microwave drying, the changes of temperature in GBR tend to fluctuating increase, which stimulated the enhancement of GAD activity in GBR and promoted the production of GABA. In microwave drying for GBR, the decrease in moisture content of GBR may hinder the synthesis of GABA. And the increase in temperature reduces the GAD activity in the GBR, and the concentration of substrate Glu or the concentration of coenzyme is insufficient, and the internal GABA synthesis capacity of GBR is weakened. In addition, pyruvate transaminase in GBR can cause the degradation of GABA due to succinic semialdehyde.

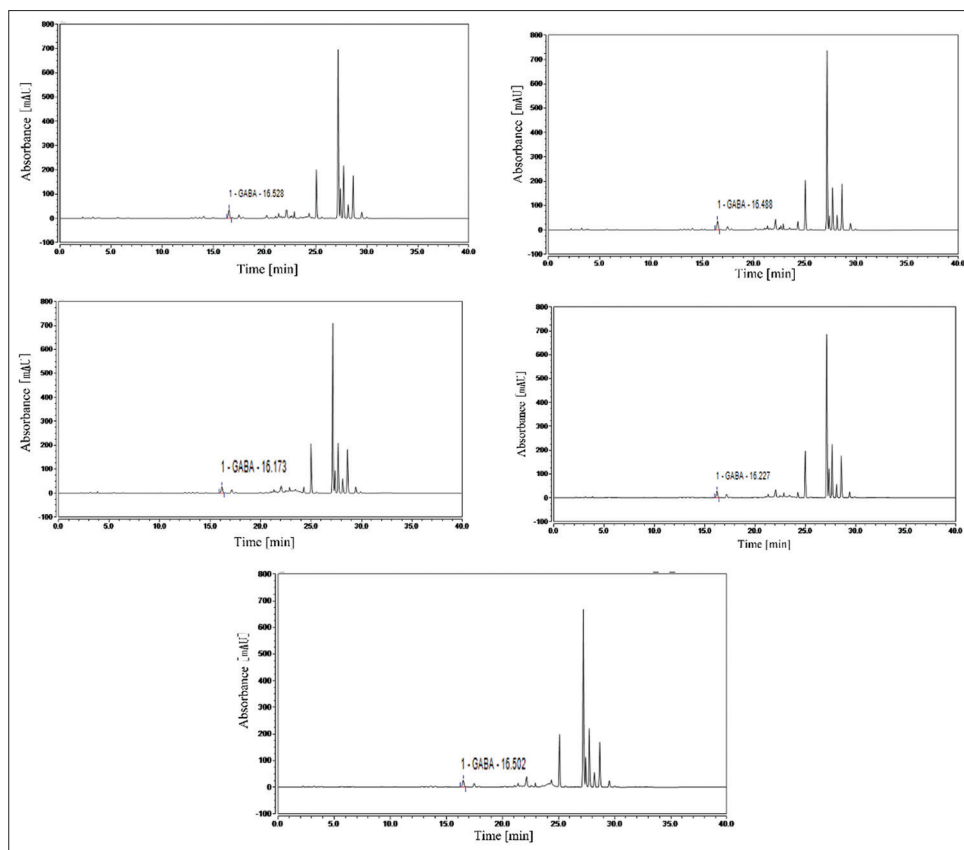


Fig 7. Liquid phase chromatogram of raw sample (a) and different drying stages for GBR.

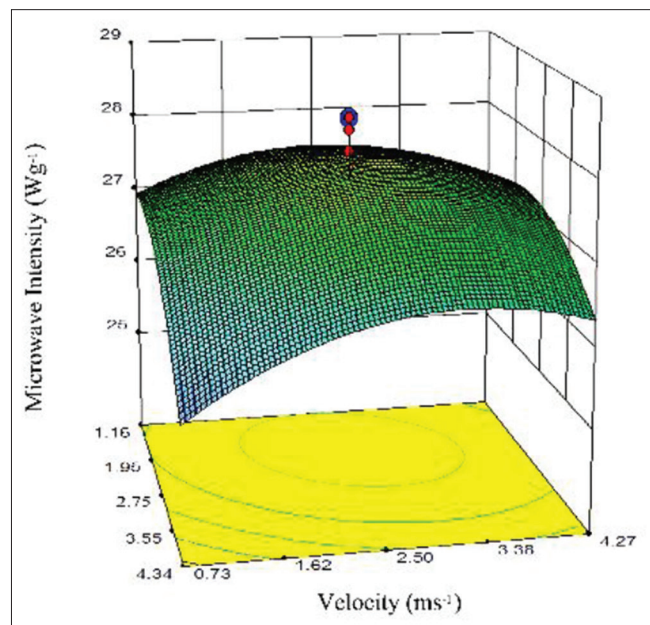


Fig 8. Effects of microwave intensity and apparent wind speed on the color of the dried GBR.

### Color change of GBR during microwave drying

The color change of GBR during drying is related to browning reaction, which depends on the drying methods and conditions. In the drying process, the drying conditions

have different degrees of influence on the color of the material, and the colors of the dried products after different drying processes treatment (drying methods and parameters) are quite different.

The dried GBR named as “golden rice” from the yellowish color, transparent appearance. It improves the market value of GBR and is recognized by consumers as the quality goal pursued by GBR manufacturers. The appearance of GBR dried by microwave present the transparency and golden color was presented in Table 2.

Table 2 shows the color difference values of GBR at different stages during the microwave drying process. As the microwave drying process proceeds, the temperature of the GBR increases and the moisture content decreases, indicating that the brightness change “*L*” of the GBR shows a downward trend. The result is attributed to the surface of GBR in weak perception; the increase of redness “*a*” value of GBR indicate that the temperature of GBR increased rapidly (15°C/min) at the beginning of drying. In microwave drying, obvious browning occurs for the GBR with higher moisture content. With the reduction of the moisture content of the GBR, its reddish browning tends to fade; the “*b*” value, indicating the yellowness of GBR, is severely browned due to high temperature. According

to the above-mentioned results, the characteristic color of golden yellow of GBR depends on the  $b$  value, and the GBR dried with high  $b$  value has the golden yellow as favorable appearance.

During the microwave drying process, the temperature of GBR increased from room temperature to 100°C in 90-120 min. The increase in temperature caused non-enzymatic reductive sugars and amino acids in GBR. Browning reaction (Maillard reaction), the reaction between the amino-containing compound (from the amino acid obtained from the enzymatic hydrolysis of the protein in the germinated rice) and the carbonyl-containing compound (from the reducing sugar obtained from the enzymatic hydrolysis of the carbohydrate in GBR), occurs to cause the reaction of the germination of rice color deepened.

The Maillard reaction process is complicated as three stages. In initial stage I, carbonyl condensation and molecular rearrangement. The first step of the carbonyl ammonium reaction is the condensation between amino-containing compounds and carbonyl-containing compounds to form Schiff, followed by cyclized into N-glucosylamine, and then rearranged by Amadori molecules to generated fructosamine, which further condenses with single molecule glucose to generated double fructosamine. In intermediate stage II, the process of further degradation of fructosamine after rearrangement. Fructosamine is dehydrated to produce hydroxymethyl furfural, which leads to browning reaction after accumulation. At same time, Fructosamine rearranges to form reductive ketones, which are unstable and condensed with ammonia compounds after further dehydration. Then, Amino acid interacts with dicarbonyl compounds. In termination stage III, Aldol condensation and polymerization form brown pigment (Sun, 2016). With the increase of reaction temperature and time, increasing amounts of free radicals accelerate the reaction rate (Liu et al., 2013). Microwave volumetric heating quickly rising temperature could generate the golden brown GBR.

#### The Optimization of microwave drying conditions considering drying quality of final GBR

The ranges of microwave drying process parameters of GBR limited in this experiment (as shown in Table 1) are as microwave intensity of 0.5-5.0 W/g, surface velocity of 0-5.0m/s, and drying duration of 2.0-10 min.  $b$  value of dried GBR increases. The results indicate that the formation of golden yellow is significantly affected by microwave intensity and apparent velocity ( $p < 0.05$ ), and microwave intensity ( $F = 7.28$ ) has the higher affects intensity than that of surface velocity ( $F = 1.29$ ). According to Fig. 8 that the  $b$  value of GBR first increases and then decreases with the

increase of the microwave intensity and apparent velocity. The statistical results in Table 3 show that the influence of these factors is significant. This phenomenon may be due to browning reaction of the GBR during drying. Temperature, moisture and oxygen play the key role to determine the browning of GBR materials. During the microwave drying process, the increase of microwave intensity can increase the temperature of the GBR, and the increase of the surface velocity can increase the airflow amount to present sufficient oxygen. In combination with appropriate moisture content, it can promote the formation of browning, resulting in the value of  $b$  increases; however, excessively high microwave intensity and apparent velocity accelerate moisture evaporation in GBR till low moisture content, which is not conducive to the formation of browning reaction with low  $b$ -value. According to the experiment results in Table 2, the formation conditions of golden yellow with high  $b$  value appeared on the surface of GBR at the microwave intensity of 2.75 W/g and the apparent velocity of 2.50 m/s.

## CONCLUSION

- (1) Microwave drying process may improve the Gamma-Aminobutyric Acid (GABA) in dried GBR due to microwave volumetric heating inducing the GAD activity. In microwave drying, the high temperature inactivation of GAD and low moisture content was not conducive to GABA synthesis of GBR.
- (2) The browning generation of dried GBR depends on the temperature, moisture and oxygen. Premium quality of dried GBR with golden appearance is achieved at high microwave intensity improving the temperature of the GBR and surface velocity bringing sufficient oxygen.
- (3) The optimal microwave drying parameters are developed as the microwave intensity of 2.75 W/g and the apparent velocity of 2.50 m/s considering the drying efficiency and quality of dried GBR.

## AUTHOR CONTRIBUTIONS

Sun Peng: formal analysis, methodology, supervision, writing original draft  
 Xu Caihua: evidence collection, validation  
 Xu Feng: investigation, writing-review  
 Chen Wudong: methodology, visualization  
 Bi Jifu: methodology, formal analysis  
 Han Changsheng: conceptualization, formal analysis  
 Tong Tong: methodology, validation  
 Liu Chenghai: conceptualization, formal analysis, supervision, funding acquisition

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