Enzymatic Preparation and Structure-activity Relationship of Sesaminol

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Abstract: As a valuable natural antioxidant, sesaminol can be used in food and medicine industries, but it is trace in sesame seeds and oil, and it is feasible to prepare sesaminol from sesaminol triglucoside (STG) which is abundant in defatted sesame cake. Therefore, in order to establish an effective enzymatic preparation method and elucidate the antioxidant structure-activity relationship of sesaminol, a suitable glycosidase for preparing sesaminol from STG were screened, enzymatic hydrolysis was optimized by single-factor test and response surface methodology, and finally, the structure-activity relationship of sesaminol was illustrated by comparative molecular field analysis (CoMFA). These results suggested that β -galactosidase was the optimal glycosidase for enzymatic hydrolysis of STG to prepare sesaminol. Under the optimal conditions of a reaction temperature of 50°C, reaction time of 4.0 h, pH of 5.5, substrate concentration of 1.0 mg/mL, and enzyme dosage of 20 mg/mL, the conversion rate of sesaminol was 98.88±0.67%. Sesaminol displayed excellent antioxidant ability in 2,2-diphenyl-1-picrylhydrazyl (DPPH, IC₅₀ = 0.0011 mg/mL), 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonate) (ABTS, IC₅₀ = 0.0021 mg/mL) radical scavenging activities and Ferric reducing antioxidant power (FRAP, 103.2998 mol/g) compared to other sesaminol derivatives. According to $-\log(\text{IC}_{50} \text{ of DPPH})$ and $-\log(\text{IC}_{50} \text{ of ABTS})$, CoMFA models were successfully established based on $Q_2 > 0.5$ ($Q_{\text{DPPH}}^2 = 0.558$, $Q_{\text{ABTS}}^2 = 0.534$). The active site of sesaminol tended to be located on the hydroxyl group of the benzene ring (R₁ position). A positive correlation between the bulky and positively charged groups at the 1H, 3H-furo [3, 4-c] furan group, the small, negatively charged groups at the R₁ position and the antioxidant activity of sesaminol. This study provides an effective method to prepare sesaminol, reveals the structure-activity relationship of sesaminol and provides theoretical basis to design the novel compound.

Key words: sesaminol triglycoside, sesaminol, enzymatic hydrolysis, antioxidant activity, structure-activity relationship

1 Introduction

Oxidative stress occurs when the amount of free radicals such as superoxide, hydrogen peroxide and nitric oxide exceeds the ability of cells to remove them through antioxidant defence mechanisms¹. It is well known that oxidative stress can accelerate ageing and lead to the occurrence and development of inflammation, hepatocarcinogenesis and degenerative diseases in elderly individuals²⁻⁴. Some antioxidant additives may be critical for the alleviation of stress-related diseases^{5, 6}. Although synthetic antioxidants such as TBHQ and BHA have been commonly added to food, there is growing anxiety about synthetic antioxidants

due to the risk of carcinogenic and liver damage⁷. Recent studies have focused on developing and utilizing antioxidants from edible natural sources⁸.

Sesame (*Sesamum indicum* L) is an important oilseed worldwide. It is one of the most popular health oilseeds due to its lignan derivatives, such as sesamin, sesamol, sesaminol and their glycosides⁹. These compounds benefit human health, exhibiting antioxidant, anti-ageing, and anti-inflammatory properties, among others¹⁰⁻¹². Among the lignans of sesame, sesaminol has shown more potent antioxidant activity *in vitro* experimental system than α -tocopherol and BHT¹³. In addition, sesaminol can

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improve the usability of tocopherols in organisms and inhibit oxidative modification in DNA¹⁴). Therefore, sesaminol is considered one of the most valuable natural antioxidants. However, few studies have been carried out on the antioxidant activity of sesaminol compared with that of other natural antioxidants, such as flavonoids and polyphenols^{15, 16}). Moreover, little attention has been given to relevant studies of sesaminol and its structure-activity relationship. As a naturally occurring trace compound, the content of sesaminol is low in sesame seeds and oil and is difficult to synthesize, which limits relevant study and application¹³).

Some researchers separated sesaminol from roasted sesame oil or refined sesame oil^{17, 18}), sesaminol content was no more than 2 mg/100 g and 10 mg/100 g in sesame seed and sesame oil respectively. This method was unsatisfactory due to low yield and reduction in the nutrition and edible values of sesame oil. Other researchers have prepared sesaminol from sesamol by chemical reactions in organic medium¹⁹). However, residues of organic solvents are a risk in the application of the food and pharmaceutical industry. Some attempts have been made to prepare sesaminol from water-soluble sesaminol triglycoside (STG) by enzymes. STG can be effectively extracted from defatted cake, which is a rich byproduct of sesame oil industries. However, low yield and long reaction time limit the preparation of sesaminol by enzymes. Peng *et al.*²⁰) reported that the yield of sesaminol prepared by enzymatic hydrolysis of STG for 24 h was only 48.9%. Therefore, it is urgent to solve the problem of the time-consuming and low conversion rate of sesaminol by optimizing tool enzyme and hydrolysis conditions. Moreover, previous research has not considered the degradation rule of STG. STG transforms into either sesaminol directly or sesaminol diglycoside (SDG) or sesaminol monoglycoside (SMG) indirectly during enzymatic hydrolysis, and SDG or SMG becomes sesaminol via further hydrolysis.

In this study, a suitable enzyme was selected from common glycosidases, and then its enzymatic conditions for the preparation of sesaminol from STG were optimized by a single-factor experimental design and the response surface method (RSM). The transformation rules of SMG and SDG were analysed. Moreover, comparative molecular field analysis (CoMFA) was built to clarify the structure-activity relationship of sesaminol.

2 Experimental Procedures

2.1 Materials

Sesame seeds were obtained from a local supermarket. STG, SDG, SMG and sesaminol were prepared in laboratory. Sesamin, sesamol, sesamol, asarinin, paulownin, cellulase (0.7 U/mg), β -glucanase (2.0 U/mg), β -glucosidase (2.0 U/

mg), β -galactosidase (8.0 U/mg), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and methanol of HPLC grade were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methylphosphogonanone A and methylphosphogonanone A were obtained from Chengdu Prifa Technology Co., Ltd. (Chengdu, China). Without additional statements, all other chemicals were purchased from Sinochem (Shanghai, China).

2.2 Preparation of STG, SDG, SMG and sesaminol

STG was isolated from defatted sesame cake according to Zhu *et al.*'s method²¹) with some modifications. Defatted sesame cake was extracted with 75% ethanol (v/v) at a ratio of 1:8 (w/v) and stirred at 20°C for 8 h 3 times. The supernatant was merged, concentrated and lyophilized to obtain a crude extract rich in STG. 2 g crude extract was dissolved in distilled water (10 mL), and then the solution was added to an open column (5.0 × 50 cm) package with polyamide (100-200 mesh). Distilled water as the mobile phase eluted the column and flowing at 1.0 mL/min. The eluate was collected with an automatic DBS 100 collector (Huxi Analysis Instrument Factory Co., Ltd., Shanghai, China) in 10 mL portions and tested with HPLC and LC-ESI/MS. According to the determined results, the fraction was collected and freeze-dried to achieve purified STG.

The preparation methods of SDG, SMG and sesaminol were developed according to the results in this study. SDG and SMG were prepared from STG by β -galactosidase, and their enzymatic hydrolysis conditions were the same except for the pH. Two amounts of 50 mg STG were dissolved in a 25 mL of 0.05 mol/L citrate buffers at a pH of 3.0 and a 0.05 mol/L phosphoric acid buffer at a pH of 7.0. SDG and SMG were prepared by enzymatic hydrolysis under the same reaction conditions of 50°C, an enzyme dosage of 20 mg/mL and a reaction time of 3.0 h, and then the reaction solutions were boiled for 5 min and centrifuged at 12000 rpm for 15 min. The supernatants were collected and loaded onto a Sephadex LH-20 column (2.0 × 50 cm). Distilled water was used as the eluent, and the flow rate was 1.0 mL/min. The eluate was assembled in 10 mL increments with an automatic collector, and the fractions containing SDG/SMG were tested, gathered and lyophilized.

Sesaminol was also prepared from STG by β -galactosidase, and the enzymatic hydrolysis conditions were the same as the enzymatic hydrolysis conditions of SDG/SMG except that the pH was 5.5. After the reaction was completed, the reaction mixture was centrifuged, 50 mL ultrapure water was added to the precipitate, and then the mixture was shaken for 2 min and centrifuged again at 12000 rpm for 15 min. Then, 50 mL ethanol was added to the precipitate, the mixture solution was shaken for 2 min and separated by centrifugation, and the supernatant was

collected. This step was repeated 3 times. All the supernatants were combined and enriched by rotary evaporation. After vacuum freeze-drying for 36 h, sesaminol was tested and collected.

2.3 Determination and identification of STG, SDG, SMG and sesaminol

The analysis of STG, SDG, SMG and sesaminol was performed on a Thermo Fisher Scientific Ultimate 3000 series HPLC system (Thermo Fisher Scientific, Waltham, MA, USA), a UV detector and an Agilent 5 TC-C18(2) (250 mm × 4.6 mm, 5 μm) column (Agilent, CA, USA). The temperature of column oven is set at 30°C. The mobile phase are methanol (solvent A) and water (solvent B), the gradient system is as follows: 0–10 min (1 mL/min, 40% A); 15–30 min (0.8 mL/min, 40% A); 40–50 min (1 mL/min, 80% A), 55 min (1 mL/min, 80% A) and 60 min (1 mL/min, 40% A). The absorbance was monitored at 287 nm for STG, SDG and SMG and 293 nm for sesaminol. The injection volume was 5 μL.

The molecular weights of STG, SDG, SMG and sesaminol were identified by LC-ESI/MS. LC-ESI/MS analysis was carried out by using an Ultimate 3000-Q Exactive-Orbitrap (Thermo, MA, USA) and a positive model.

2.4 Selection of optimum enzyme for preparation of sesaminol and qualitative analysis of enzymatic hydrolysis products

According to related literature^{22, 23)}, cellulase, β-glucanase, β-glucosidase and β-galactosidase were tested in the experiment, and their optimum conditions are shown in Table 1. 10 mg STG was mixed with 10 mL buffer solution. Glycosidase was added to the reaction solution at the same amount of enzyme activity. The reaction solution was shaken in a water bath for 8.0 h at the optimal temperature. The sample was boiled for 5 min followed by centrifugation at 10,000 × g for 20 min at 4°C and filtered through a 0.22 μm microfiltration membrane. HPLC qualitatively analysed the composition of the hydrolysate. The HPLC method was in line with the method described in 2.2. A suitable glycosidase was selected based on the retention rate of STG (calculated by (1)) and hydrolysate yields (calculated by (2)).

$$Y_1 = \frac{m_2}{m_1} \times 100\%, \quad (1)$$

$$Y_2 = \frac{m_3 M_1}{m_1 M_2} \times 100\%, \quad (2)$$

In equations (1) and (2), Y_1 represents the retention rate of STG, Y_2 represents the conversion rate of enzymatic hydrolysis products, m_1 represents the weight of STG in the reaction solution at 0 h, m_2 represents the weight of STG in the reaction solution at the end of the reaction, m_3 represents the weight of sesaminol in the reaction solution at the end of the reaction, M_1 represents the molecular weight of STG, and M_2 represents the molecular weight of enzymatic hydrolysis products.

2.5 Optimization of the enzymatic hydrolysis conditions

The optimal conditions for the preparation of sesaminol from STG were determined by a single-factor test and RSM^{24, 25)}. Reaction time, temperature, pH, substrate concentration and enzyme concentration were selected as variables in the single-factor test of the experimental design. The levels of these five factors were set as follows: reaction times of 0.5–12.0 h; temperatures of 20–60°C; pH of 3.0–7.0; substrate concentration of 1.0–3.0 mg/mL; and enzyme concentration of 10–30 mg.

According to the results of the single-factor test, pH (x_1), temperature (x_2) and reaction time (x_3) were chosen for optimization by RSM. The levels of independent variables are shown in Table 2.

2.6 DPPH free radical scavenging activity assays

The abilities of 10 compounds to scavenge DPPH free radicals were assessed according to the method of Yeo *et al.*²⁶⁾. First, 100 μL samples of different concentrations were added to a 96-well microplate, and 100 μL DPPH in 80% ethanol (0.2 mmol/L) was prepared in each well and mixed through the flat plate oscillation period. The mixture was incubated at room temperature for 30 min in the dark. The absorbance was measured using an Infinite M Nano absorbance plate reader (Tecan Group Ltd., Zürich, Switzerland) at 517 nm. The DPPH radical scavenging capacity was calculated using the following equation:

$$\text{DPPH}_{\text{scavenging ability}} = \left(1 - \frac{A_{\text{sample}} - A_{\text{sample background}}}{A_{\text{control}}} \right) \times 100\% \quad (3)$$

where A_{sample} is the absorbance of the sample and DPPH,

Table 1 The optimum conditions of the enzymatic hydrolysis of STG by four enzymes.

Enzyme	Temperature/°C	pH	Time/h	Dosage/mg/mL
β-galactosidase	50.0	5.0	8.0	15.0
β-glucosidase	50.0	5.0	8.0	4.0
β-glucanase	37.0	5.0	8.0	2.4
Cellulase	55.0	4.6	8.0	171.4

Table 2 Coded levels and actual values (in parentheses) of the variables in Box–Behnken design.

Run	x ₁	x ₂	x ₃	Y (%)
1	− 1 (4.5)	− 1 (45.0)	0 (4.0)	79.24
2	− 1 (4.5)	1 (55.0)	0 (4.0)	77.12
3	1 (5.5)	− 1 (45.0)	0 (4.0)	92.56
4	1 (5.5)	1 (55.0)	0 (4.0)	92.63
5	0 (5.0)	− 1 (45.0)	− 1 (3.5)	85.78
6	0 (5.0)	− 1 (45.0)	1 (4.5)	85.92
7	0 (5.0)	1 (55.0)	− 1 (3.5)	84.13
8	0 (5.0)	1 (55.0)	1 (4.5)	84.21
9	− 1 (4.5)	0 (50.0)	− 1 (3.5)	83.89
10	1 (5.5)	0 (50.0)	− 1 (3.5)	98.88
11	− 1 (4.5)	0 (50.0)	1 (4.5)	84.13
12	1 (5.5)	0 (50.0)	1 (4.5)	96.73
13	0 (5.0)	0 (50.0)	0 (4.0)	95.94
14	0 (5.0)	0 (50.0)	0 (4.0)	95.62
15	0 (5.0)	0 (50.0)	0 (4.0)	95.88

A_{sample background} is the absorbance of the sample background (sample and ethanol instead of DPPH), and A_{control} is the absorbance of the control (DPPH and 100 µL of ethanol instead of sample).

2.7 ABTS free radical scavenging activity assays

The method of Re *et al.* was used to determine ABTS assay²⁷. 20 µL sample solution and 200 µL of working solution were added to the 96-well plate. The mixture was then mixed on the plate vibrator and reacted for 6 min in the dark at room temperature. An Infinite M Nano absorbance plate reader was used to observe the absorbance of the sample solution at 734 nm. The sample solution with a certain gradient concentration was used to determine the ABTS scavenging activity. The IC₅₀ value was calculated by SPSS analysis.

$$\text{ABTS}_{\text{scavenging ability}} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100 \quad (4)$$

where A_{control} is the absorbance of methanol and ABTS radicals and A_{sample} is the absorbance of ABTS radicals in the sample.

2.8 Ferric reducing antioxidant power (FRAP)

The FRAP capacity was estimated following Benzie *et al.*²⁸ as described with slight modification. Fresh FRAP reagent (150 µL) was pipetted into a well of a 96-well plate and mixed with 30 µL of 1 mg/mL sample solution. The absorbance of the blank sample (150 µL FRAP reagent and 30 µL distilled water) was recorded after incubation for 30 min at 37°C. A standard curve was plotted by measuring the absorbance of 0–2 mmol FeSO₄ at 593 nm. The results are

presented in µmol FeEq/g of sample, according to a standard curve of FeSO₄.

2.9 3D-QSAR of sesaminol

3D-QSAR helps to gain insight into the relationship between specific bioactivity and molecular structure. As a classic 3D-QSAR technique, CoMFA illuminates the effect of steric and electrostatic fields of a set of aligned compounds on their biological activity by associating these 3D fields with the corresponding experimental activities of ligands interacting with a common target receptor²⁹. CoMFA has been applied to demonstrate the structure-activity relationships³⁰.

Sesaminol, STG, SDG, SMG, asarinin, sesamin, sesamol, paulownin, methylophipogon flavone A and methylophipogonanone A were utilized to construct the CoMFA model by SYBYL-X 2.1.1 software. The molecular structures of the 10 compounds were drawn with a sketch module and energy minimization using a conjugate-gradient minimizer. The MMFF94 method was used to calculate the partial atomic charges of all the compounds, and then the Powell conjugated gradient algorithm method was utilized to optimize their geometry. Moreover, 0.0005 kcal/(mol Å) was selected as the energy convergence criterion, and the maximum iteration was set as 10,000 to construct a steady conformation⁷.

To construct the CoMFA model based on the pIC₅₀ of DPPH, sesaminol was chosen as a template molecule in the molecule alignment stage because it is the strongest DPPH scavenging compound; other compounds were aligned on the framework structure of 1,3-benzodioxole in the tem-

plate molecule. To build the CoMFA model based on the pIC_{50} of ABTS, methylophiopogonanone A was selected as the template molecule in the molecule alignment stage because it is the strongest ABTS scavenging compound (Fig. 4(a)), another operation is to refer to the method of the CoMFA model based on the pIC_{50} of DPPH.

The linear correlation between the biological activity and the CoMFA model was assessed by Partial least squares (PLS) analysis. In this study, leave-one-out (LOO) cross-validation procedures were used to determine the optimal number of components and cross-validation correlation coefficient (Q^2), while non-cross-validated analysis was used to calculate the non-cross-validated correlation coefficient (R^2), standard error of estimate (ESS), and F-value³¹. The CoMFA model was regarded as acceptable when Q^2 and R^2 were greater than 0.5 and 0.6, respectively³⁰.

2.10 Statistical analysis

All the tests were performed in triplicate. The statistical significance was determined by the level of $p < 0.05$. There was no significant difference between the values with the same letters in one curve. SAS software (Version 8.5, North Carolina, USA) and SPSS software (version 20.0; SPSS, Chicago, IL, USA) were applied to the experimental design and statistical analysis.

3 Results and Discussion

3.1 Preparation and identification of STG, SDG, SMG and sesaminol

STG, SDG, SMG and sesaminol were successfully obtained by column chromatography and liquid-liquid extraction. Their purity was determined by HPLC to be 98.17%, 98.19%, 98.53% and 99.35%, respectively. The contents of the solution mixture were also determined by HPLC, including the levels of STG, SDG, SMG and sesaminol. The HPLC chromatogram is shown in Fig. 1A. As shown in Figs. 1B-1E, fragments at m/z 879.2479, 717.1979, 555.1470 and 393.0940 were the $[M + Na]^+$ ions, and the molecular weights of the corresponding compounds were 856.2 581, 694.2 081, 532.1 572 and 370.1 042, which were in agreement with previous reports^{24, 32}. The results showed that STG could be obtained from sesame cake. STG could be a raw material that can be used for the preparation of SMG, SDG and sesaminol. Otherwise, the four compounds obtained could be used in the following test as standard and raw material.

3.2 Effect of different enzymes on the conversion rate of sesaminol

As shown in Fig. 2A, β -galactosidase, β -glucosidase, β -glucanase and cellulase were reacted with STG under the optimal conditions for 8.0 h. Sesaminol and SDG were the

main products, and their final conversion rates were as follows: the sesaminol exhibited conversion rates of 94.62%, 5.16%, 1.86%, 4.04% while SDG exhibited rates of 0%, 85.84%, 78.21%, 86.15% in the reactions with the four different enzymes. The highest conversion rate of sesaminol appeared in the reaction involving β -galactosidase hydrolysate. However, most of the STG was converted to SDG by β -glucosidase, β -glucanase and cellulase. These results depend on the substrate structure and cleavage specificity of the enzyme. The structure of the sesaminol glycoside was sesaminol 2'-O- β -D-glucopyranosyl-(1-2)-O-[β -D-glucopyranosyl-(1-6)]- β -D-glucopyranoside (STG)²¹. β -Galactosidase is an exoglycosidase that can degrade β -glycosidic bonds, including β -(1-2)-glycosidic bonds, β -(1-6)-glycosidic bonds, and β -(1-4)-glycosidic bonds²⁵. However, β -glucosidase, β -glucanase and cellulase preferentially cleave glycosidic bonds to terminal nonreducing residues in β -glucosides, β -(1-6)-glycosidic bonds and β -(1-4)-glycosidic bonds, respectively^{33, 34}. β -Galactosidase showed higher performance in the preparation of sesaminol by hydrolysing STG than the other enzymes³⁵. According to the results, it can be inferred that β -galactosidase was suitable to prepare sesaminol from STG effectively.

3.3 Effect of reaction time on the conversion rate of sesaminol

The retention of STG and the conversion of its hydrolysis products are shown in Fig. 2B. A dramatic change in the content of each component occurred in the first 1.0 h during enzymatic hydrolysis. The STG rapidly disappeared after 0.5 h. The SDG conversion rate reached a peak at 84.69% at 0.25 h and then decreased to 0% at 1.0 h. The conversion rate of SMG reached a maximum of 19.91% at 0.5 h and subsequently gradually disappeared at 3.0 h. The conversion rate of sesaminol significantly increased to 84.15% within 1.0 h and continued to increase to a high point at 4.0 h. Therefore, the reaction path from STG to sesaminol included two intermediate products, SDG and SMG, glucose. The study results were consistent with those of Nair *et al.*³⁶. The conversion rate of sesaminol did not significantly increase over an 8 h period. Thus, 4.0 h was selected as the proper reaction time. The reaction takes less time than those using β -glucosidase and microbial fermentation to prepared sesaminol^{13, 20, 21}.

3.4 Effect of reaction temperature on the conversion rate of sesaminol

The effect of temperature (20-60°C) on the conversion rate of sesaminol from STG (1.5 mg/mL) by β -galactosidase at pH 5.0 after incubation for 4 h is presented in Fig. 2C. The STG and SDG disappeared at the end of the reaction. The lowest conversion rate of sesaminol was 29.4% at 20°C. At the same temperature, the SMG conversion rate reached 69.0%. As the reaction temperature gradually in-

Fig. 1 HPLC chromatograms analysis of STG and its hydrolysis products (A, Peak 1, STG; Peak 2, SDG; Peak 3, SMG; Peak 4, Sesaminol); ESI-MS spectra of STG and its hydrolysis products (B, peak 1, STG, m/z 879.2479, $[M + Na]^+$; C, peak 2, SDG, m/z 717.1979, $[M + Na]^+$; D, peak 3, SMG, m/z 555.1470, $[M + Na]^+$; E, peak 4, sesaminol, m/z 393.0940, $[M + Na]^+$).

creased to 50°C, the conversion rate of sesaminol significantly increased to a maximum of 95.3% ($p < 0.05$), while the conversion rate of SMG decreased to a minimum of 3.0%. The conversion rate of sesaminol significantly decreased as the temperature exceeded 60°C, which was related to the enzyme denaturation caused by high tem-

perature. This result was consistent with the report that the optimum temperature of β -galactosidase from *Aspergillus oryzae* was in the range of 45 to 55°C³³. Therefore, 50°C was selected as the optimum reaction temperature.

Fig. 2 Effect of different enzymes (A), reaction time (B), reaction temperature (C), enzyme dosage (D), substrate concentration (E) and pH (F) on the conversion rate of sesaminol. (Different lowercase letters on different columns indicate significant difference ($p < 0.05$).)

3.5 Effect of enzyme dosage on the conversion rate of sesaminol

The influence of the enzyme dosage on the conversion rate of sesaminol is shown in Fig. 2D. When the enzyme dosage was increased from 10 mg/mL to 20 mg/mL, the STG and SDG disappeared, the conversion rate of SMG ranged from 21.3% to 2.7%, and the conversion rate of

sesaminol significantly increased to 95.4%. However, when the enzyme dosage increased from 20 mg/mL to 30 mg/mL, the conversion rate of sesaminol did not significantly change. Considering the cost of the enzyme, the enzyme dosage was set at 20.0 mg/mL.

3.6 Effect of substrate concentration on the conversion rate of sesaminol

Substrate concentration is one of the main affecting factors for enzymatic hydrolysis reaction³⁷⁾. As shown in Fig. 2E, when the substrate concentration was 1.0 mg/mL, STG was completely enzymatically hydrolysed, and the conversion rate of sesaminol was up to 95.8% with a small amount of residual SMG. As the substrate concentration increased, the conversion rate of sesaminol significantly decreased, and the conversion rates of the SDG and SMG slightly increased. Overall, these results indicate that 1.0 mg/mL was the optimal substrate concentration.

3.7 Effect of reaction pH on the conversion rate of sesaminol

As shown in Fig. 2F, the conversion rate of sesaminol ranged from 7.2% to 95.9% as the pH increased from 3.0 to 5.0. There was no significant difference between the conversion rate of sesaminol at pH 5.0 and 6.0 ($p > 0.05$). However, the conversion rate of sesaminol remarkably decreased at pH 7.0 ($p < 0.05$). The highest conversion rates of SMG and SDG were 74.2% at pH 7.0 and 79.6% at pH 3.0. Therefore, it was suitable for enzymatic hydrolysis of STG into sesaminol at pH 5.0-6.0. The optimal pH for sesaminol production with β -galactosidase was in agreement with the optimal pH range of 5.0-6.0 for ginsenoside Rg(3) hydrolysis³⁸⁾.

3.8 Response surface experimental results

Three factors and three levels Box-Behnken design in-

cluding 15 trials was used to assess the effect of hydrolysis factors, including pH (x_1), temperature (x_2) and reaction time (x_3), on the conversion rate of sesaminol; the results of these trials were used to optimize these factors. The experimental results are shown in Table 2. The experimental data were analysed by multiple regression analysis, and a second-order polynomial Eq. (5) was used to describe the relationship between the independent variables and survey the conversion rate of sesaminol. The second-order equation (in coded format) was presented:

$$Y = 95.813 + 7.052 x_1 - 0.676 x_2 + 0.211 x_3 - 2.264 x_1^2 + 0.547 x_1 x_2 - 0.597 x_1 x_3 - 8.161 x_2^2 - 0.015 x_2 x_3 - 2.641 x_3^2 \quad (5)$$

In Table 3 above, we can see the ANOVA results. The Model F-value was 362.8777. A p -value lower than 0.0001 indicates that the model was in line with the actual data. The determination coefficient (R^2) was 0.9985, which suggested that only 0.15% of the total variation was not fitted with the model. The Lack of Fit was 0.082943 (> 0.05), indicating that the lack of fit was not significant. The p -values of the linear coefficient (x_1, x_2) and quadratic coefficients (x_1^2, x_2^2, x_3^2) were less than 0.05, implying the significant effects of these factors on the conversion rate.

3.8.1 Response surface three-dimensional plots

To illustrate the intercorrelations between the three above factors and the conversion rate of sesaminol, a three-dimensional response surface was built (Fig. 3). When the effect of two factors was plotted, the other factor was

Table 3 ANOVA for response surface quadratic model.

Source	Sum of squares	DF	Mean square	F-value	Probability	Significance
Model	673.8803	9	74.875 59	362.877 7	0.000 1	**
x_1	397.902	1	397.902	192 8.396	0.000 1	**
x_2	3.658 513	1	3.658 513	17.730 65	0.008 402	**
x_3	0.357 013	1	0.357 013	1.730 229	0.245 469	
$x_1 * x_2$	1.199 025	1	1.199 025	5.810 966	0.060819	
$x_1 * x_3$	1.428 025	1	1.428 025	6.920 794	0.060 812	
$x_2 * x_3$	0.000 9	1	0.000 9	0.004 362	0.949 902	
x_1^2	18.928 43	1	18.928 43	91.734 93	0.000 21	**
x_2^2	245.955	1	245.955	1191.998	0.000 1	**
x_3^2	25.766 41	1	25.766 41	124.874 6	0.000 1	**
Residual	1.031692	5	0.206 338			
Lack of fit	0.973 825	3	0.324 608	11.219 18	0.082 943	
Pure error	0.057 867	2	0.028 933			
Cor total	674.912	14				
$R^2 = 0.998 5$				$R^2_{Adj} = 0.995 7$		

(Note: *Significant difference ($p < 0.05$); **Remarkable difference ($p < 0.01$))

Fig. 3 The effect of pH and temperature interaction on the conversion rate of sesaminol at different levels.

fixed. In the investigation range, pH and temperature were significant factors, so the curve of the response surface and contour map of the conversion rate of sesaminol at fixed reaction times were observed. **Figures 3A and 3B** shows that as the reaction temperature was fixed, and that the conversion rate of sesaminol gradually increased and then tended to be stable with increasing pH. The enzyme activity remained stable because the pH was near the optimum pH range for the enzyme. This result was in line with the single-factor test studies. When pH was the fixed variable, the conversion rate of sesaminol increased when the reaction temperature was raised from 45°C to 50°C and then decreased when the reaction temperature was raised from 50°C to 55°C because high temperature leads to enzyme denaturation, so enzyme activity decreased^(38, 39). The trend of sesaminol conversion at 4.0 h and 4.5 h was similar to that at 3.5 h (**Figs. 3C-3F**).

3.8.2 Verification of optimization

The optimum conditions predicted by SAS software were as follows: the pH value was 5.67, the reaction temperature was 49.66°C, the reaction time was 3.91 h, the substrate concentration was 1.0 mg/mL, and the enzyme dosage was 20 mg/mL. Under these conditions, the conversion rate of sesaminol was $100.42 \pm 0.52\%$. For convenience of operation, the enzymatic hydrolysis process parameters were set to a pH of 5.5, temperature of 50°C, enzymatic hydrolysis time of 4.0 h, substrate concentration of 1.0 mg/mL, and an enzyme dosage of 20 mg/mL. 3 parallel tests were conducted, and the conversion rate of sesaminol was $98.88 \pm 0.67\%$. The result conformed to the predicted data. Therefore, the process parameters of the enzymatic hydrolysis of STG into sesaminol based on the response surface method are accurate and reliable. Compared with a previous report^(17, 18, 20), the performance of the enzymatic preparation of sesaminol from STG improved due to the selection of the enzyme and the optimization of the hydrolysis conditions; the conversion rate of sesaminol rose from 48.90% to 98.88%, the reaction time decreased to 1/6 of previous

reports⁽²⁰⁾. The enzymatic hydrolysis technology not only reduced the nutrition and edible values of sesame oil, but also could improve the utilization rate and added value of sesame cake, which is a rich by-product of sesame oil industries^(17, 18).

3.9 3D-QSAR of sesaminol and its derivative

The antioxidant ability of sesaminol, STG, SDG, SMG, asarinin, sesamin, sesamolin, paulownin, methylophiopogonanone A and methylophiopogonone A was measured. The results of DPPH and ABTS radical scavenging ability tests and FRPA are shown in **Table 4**. Sesaminol exhibited excellent antioxidant activity in DPPH ($IC_{50} = 0.0011$ mg/mL), ABTS ($IC_{50} = 0.0021$ mg/mL) radical scavenging activities and FRAP (103.2998 mol/g) among sesaminol derivatives, which was also in accordance with a previous study⁽⁴⁰⁾.

To elucidate the effects of sterics and electrostatic fields on the antioxidant activity of sesaminol, CoMFA models based on $-\log(IC_{50}$ of DPPH) and $-\log(IC_{50}$ of ABTS) were established (**Table 5**). As seen in **Table 6**, the CoMFA model for $-\log(IC_{50}$ of DPPH) and $-\log(IC_{50}$ of ABTS) was successfully established based on $Q^2 > 0.5$ ($Q_{DPPH}^2 = 0.558$, $Q_{ABTS}^2 = 0.534$). These models had good R^2 values ($R^2 = 1.000$) with low standard errors of estimate (< 0.05) and a high F value ($F_{DPPH} = 171737.028$, $F_{ABTS} = 8955.818$). The CoMFA based on the DPPH scavenging activity has the highest Q^2 ; moreover, it was reliable to evaluate the antioxidant activity of fat-soluble sesaminol by DPPH scavenging assay. Therefore, this paper only analysed and discussed the CoMFA model based on the DPPH activity results.

In **Figs. 4A-4B**, there are large green areas in the 1H, 3H-furo[3,4-c]furan group and C₂" and C₆" positions of the substituent R₂, indicating that the introduction of substituents with larger groups here is beneficial to the improvement of activity. This observation also agrees with the experimental result, as the larger 5,7-dihydroxy-6,8-dimethyl-4H-1-benzopyran-4-one group at the R₂ position of methylophiopogonone A presented higher DPPH inhibi-

Table 4 The antioxidant activity of sesaminol and its derivatives.

Name	IC ₅₀ ABTS (mg/mL)	IC ₅₀ DPPH (mg/mL)	FRAP (μmol/g)
sesaminol	0.0021	0.0011	103.2998
STG	31.0174	9.39E+12	56.0517
SDG	121140.9194	46707.9563	43.4433
SMG	5332.6395	598.6076	44.6696
Asarinin	57.3763	0.2128	37.0110
Sesamin	2.57E+05	3.05E+10	39.8636
Sesamolin	2.44E+06	4.79E+18	55.0709
Paulownin	34195.659	1009.6503	8.3261
Methylophiopogonanone A	0.0047	0.0097	91.3624
Methylophiopogonone A	0.0027	0.0042	98.6291

Table 5 The antioxidant activity and molecular formula of sesaminol and its derivatives.

Name	R ₁	R ₂	– LOG(IC ₅₀ of DPPH)		– LOG(IC ₅₀ of ABTS)	
			Observed	Predicted(SEM)	Observed	Predicted(SEM)
Sesaminol	–OH		2.5445	2.5410(0.0035)	2.2533	2.2270(0.0263)
STG			– 13.0400	– 13.0220(– 0.0180)	– 1.5590	– 1.5490(– 0.0100)
SDG			– 4.8279	– 4.8410(0.0131)	– 5.2418	– 5.2260(– 0.0158)
SMG			– 3.0511	– 3.0560(0.0049)	– 4.0009	– 4.0009(0.0000)
Asarinin	–H		0.2215	0.2280(– 0.0065)	– 2.2093	– 2.2070(– 0.0023)
Sesamin	–H		– 10.9346	– 10.9410(0.0064)	– 5.8610	– 5.8630(0.0020)
Sesamolin	–H		– 19.1118	– 19.1120(0.0002)	– 6.8186	– 6.8200(0.0014)
Paulownin	–H		– 3.4355	– 3.4500(0.0145)	– 4.9653	– 4.9560(– 0.0093)
Methylophiopogonanone A	–H		1.5440	1.5290(0.0150)	1.8642	1.7940(0.0702)
Methylophiopogonone A	–H		1.9085	1.9420(– 0.0335)	2.1030	2.1940(– 0.0910)

Note: Standard error of means (SEM)

tory activity than sesamin. There is a yellow region at the R₁ position, indicating that smaller groups would enhance the antioxidant activity. The smaller –OH at the R₁ position of sesaminol presented better DPPH inhibitory activity than did SMG, SDG and STG. This conclusion obeyed the scavenging mechanism of the DPPH radical; that is, the larger the steric hindrance of the compound was, the more challenging it was for to come into contact with the un-

paired electron of the intermediate N atom in the DPPH radical through the spatial barrier of the three benzene rings of DPPH²⁷⁾.

In **Figs. 4C** and **4D**, the red region appears at the R₁ position, indicating that the introduction of more electronegative groups here is conducive to the improvement of the compound activity. This finding is in line with the experimental data, as the negatively charged –OH group at the R₁

Table 6 Statistical parameters for the fitting of the CoMFA model for sesaminol and its derivatives.

Parameter	CoMFA based on –Log(IC ₅₀ of DPPH)	CoMFA based on –Log(IC ₅₀ of ABTS)
NC ¹	5	4
Q ²	0.558	0.534
R ²	1.000	1.000
F value	171 737.028	8955.818
Standard error of estimate	0.023	0.055
The contribution of steric field	0.650	0.720
The contribution of electrostatic field	0.350	0.280

NC¹ represents optimal number of principal components

Fig. 4 (a) The common structure of 10 compounds used for the alignment and the overall alignment of the molecules used in the 3D-QSAR study; (b) Steric and electrostatic contour maps of the CoMFA model of sesaminol ((A) Steric contour map of the CoMFA model based on $-\log(\text{IC}_{50}$ of DPPH), (B) Electrostatic contour map of the CoMFA model $-\log(\text{IC}_{50}$ of DPPH), (C) Steric contour map of the CoMFA model based on $-\log(\text{IC}_{50}$ of ABTS), (D) Electrostatic contourmap of the CoMFA model $-\log(\text{IC}_{50}$ of ABTS).)

position of sesaminol resulted in higher DPPH inhibitory activity than that of sesamin, whose R₁ position was occupied by the less negatively charged -H. A large blue area appeared in the 1H, 3H-furo[3,4-c]furan group of substituent R₂, indicating that the introduction of positively charged groups in the region was beneficial to the increase in the antioxidant activity of the compound. This increase in activity is because the introduction of a positive charge in this region can increase the rejection of DPPH radicals and increase the probability of DPPH radicals coming into contact with the compound from R₁ (-OH) to improve the scavenging ability of DPPH radicals. Combined with the results of the steric field and electrostatic field analysis, the data indicated that -OH at the sesaminol R₁ position

was the active centre of DPPH free radical scavenging by sesaminol, and the result was in good agreement with a previous report that found that -OH increased the activity of flavonoids¹⁶⁾.

4 Conclusions

This study investigated the preparation method and structure-activity relationship of sesaminol. The results showed that β -galactosidase was a suitable enzyme to prepare sesaminol from STG. The optimal enzymatic hydrolysis conditions were established. In addition, the transformation rules of sesaminol, SMG and SDG were investi-

gated to further reveal the mechanism of enzymatic hydrolysis. Furthermore, compared to other sesaminol derivatives, sesaminol has excellent scavenging capacity for DPPH, ABTS and FRAP. CoMFA models were developed according to $-\log(\text{IC}_{50} \text{ of DPPH})$ and $-\log(\text{IC}_{50} \text{ of ABTS})$. The active site of sesaminol tended to be located on the hydroxyl group of the benzene ring (R_1 position). This study not only provides a short-time and effective method to prepare sesaminol, reveals the structure-activity relationship of sesaminol, but also provides theoretical basis to design the novel compound. The results are expected to provide more evidence for the utility of sesame as an antioxidant functional food.

Author Contributions

Gao, J.H.: Conceptualization, Data curation, Writing-original draft, Software, Funding acquisition. Wang, R.D.: Formal analysis, Investigation, Writing-review & editing. Lu, X.: Conceptualization, Methodology, Software, Writing-review & editing. Jia, C.: Formal analysis, Resources, Software. Sun, Q.: Validation, Project administration. Huang, J.N.: Funding acquisition, Supervision, Resource. Wei, S.L.: Methodology, Software. Ma, L.: Visualization, Methodology.

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