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ORIGINAL PAPER

Preparation of Sesaminol from Sesaminol Triglucoside by β-Glucosidase and Cellulase Hydrolysis

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Abstract Sesaminol triglucoside (i.e., 2,6-*O*-di(β-D-glucopyranosyl)-β-D-glucopyranosyl sesaminol, STG) is a physiologically active substance obtained abundantly from defatted sesame cake. Since, the industrial preparation of sesaminol from STG has not been reported previously, the aim of this research was to prepare sesaminol by hydrolysis of STG using β-glucosidase and cellulase. Under the optimal conditions of total enzyme dosage 100 μL (8,000.72 U), the ratio of β-glucosidase and cellulase 20:80 (v/v) (0.72:8,000, U/U), reaction time 24 h, substrate concentration 6 mg/mL, reaction temperature 50 °C, and reaction system pH 4.8, the yield of sesaminol was 48.9 %. Further, sesaminol and other hydrolysis products (sesaminol diglucoside and sesaminol monoglucoside) were successfully determined by high performance liquid chromatography and electrospray ionization/mass spectrometry.

Keywords Sesaminol triglucoside (STG) · β-Glucosidase · Cellulase · Sesaminol

Introduction

Sesame seed (*Sesamum indicum* L.) has always been an oilseed of great importance worldwide, and it is rich in various bioactive furofuran lignans, particularly sesamin, sesamol, sesamolinol and sesaminol glucosides [1], which play many essential roles in human health due to their antioxidant, chemoprotective, anti-inflammatory, and neuroprotective potential [2–4]. Sesaminol triglucoside (STG), a water-soluble lignan, is the richest lignan glucoside in sesame seeds [5]. STG can be efficiently obtained from de-fatted sesame cake (DSC) which is an abundant by-product of the sesame oil industries [6, 7]. The content of STG in sesame seeds varies from 14.1 to 1,560 mg/100 g [2, 8], depending on sesame cultivars and processing methods [9]. Many studies have revealed that STG hardly shows oxidation resistance *in vitro* [10, 11]. However, it could be gradually hydrolyzed to produce sesaminol aglycones by microbiota in the intestines which play a critical role in inhibition of oxidative effects [12].

Undoubtedly, sesaminol aglycones produce higher biological activities (e.g., antioxidant [1] and antitumor activity [13]) than sesaminol glycosides. Sesaminol in unroasted sesame oil is obtained mainly from the conversion of sesamolin by acid catalysis during industrial bleaching process rather than from STG hydrolysis [14]. Some β-glucosidases have been reported to hydrolyze STG [2, 15–17]. Moreover, conversions of lignan glucosides to their corresponding aglycones by microbial fermentation have also been widely researched in recent years [10, 13].

Sesaminol, a natural physiologically active ingredient from STG hydrolysis, may be used as an important additive in functional foods. However, studies of the hydrolysis of STG by β-glucosidase and cellulase have not been reported yet. So, the study was conducted to produce sesaminol through hydrolysis of STG using the enzymes mentioned above. Further, the identification of sesaminol and other hydrolysis products [sesaminol diglucoside (SDG) and sesaminol monoglucoside (SMG)] was also performed using chromatographic techniques.

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Materials and Methods

De-Fatted Sesame Cake

Sesame cake, a by-product acquired from sesame oil processing, was supplied free of charge by Yicheng Silver Sea Oil Co., Ltd. (Zhumadian, China). The sesame cake was ground into powder with a pulverizer (Nine Yang Co., Ltd., Shanghai, China) after vacuum drying, passed through an 80-mesh sieve, and de-fatted thoroughly with *n*-hexane at ambient temperature. The DSC obtained was air-dried in the dark, and then stored at −20 °C. The oil contents of sesame cake and DSC on dry basis were 1.3 and 0.14 %, respectively, obtained by soaking the samples in petroleum ether (30–60 °C) in a Soxhlet apparatus (Fusisainuo Analytical Instruments Co., Ltd., Suzhou, China) [18]. Analysis of the resulting DSC indicated that the content of STG was 14.76 mg/100 g DSC.

Chemicals and Reagents

Naringenin (4′,5,7-trihydroxyflavanone >98 %), used as internal standard [8], was purchased from Nanjing Goren Bio-Technology Co., Ltd. (Nanjing, China). High performance liquid chromatography (HPLC)-grade methanol was purchased from Merck Serono Co. Ltd. (Darmstadt, Germany). Ethanol, *n*-hexane, and methanol purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) were of analytical grade. Polyamide (100 mesh) for column chromatography was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). An Automatic collector BSZ-100 was purchased from Huxi Analysis Instrument Factory Co., Ltd. (Shanghai, China).

Enzymes and Assays

β-Glycosidase (from *Aspergillus niger*, 30 mg/mL, 36 U/mL) was obtained from Sigma-Aldrich Corporate (St. Louis, USA). One unit of β-glycosidase activity corresponds to the amount of enzyme which hydrolyzes 1 μmol *p*-nitrophenyl-β-D-glucopyranoside per minute at pH 4.0 and 37 °C. Cellulase (from *Trichoderma reesei*, 100,000 U/mL) was obtained from Shandong Long Kurt Enzyme Co., Ltd. (Shandong, China). One unit of cellulase activity is equivalent of 1 mg of glucose produced by hydrolysis of carboxymethyl cellulose per hour at pH 5.0 and 55 °C. All assays were carried out in triplicate, with control groups to rectify for the background in substrate and enzyme samples [19].

Preparation of Sesaminol Triglucoside

Extraction method of STG from de-fatted sesaminol cake (DSC) was developed according to the previous reports with several modifications [6]. Briefly, for the preparation of STG, 50 g of DSC powder was extracted two times by stirring with power mixer (Shanghai Specimen Model Factory, Shanghai, China) under the following conditions: ethanol concentration 75 % (v/v), solid–liquid ratio 0.05 g/mL, temperature 25 °C, extraction time 10 h. After the supernatants were centrifuged at 3,583×*g* for 10 min, the supernatants were combined, concentrated by rotary evaporation (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China, RE-52A) under a certain reduced pressure, and lyophilized to afford the crude extract of STG. Then 1.7 g of the above crude extract was dissolved in 5 mL deionized water, filtered through a 0.45 μm microporous membrane (Teflon), and supplied to the polyamide chromatography column (1.6 × 50 cm) after previous pretreatment. This column was eluted using distilled water with flow velocity of 1.2 mL/min. Meanwhile, the elution fractions were automatically collected in 10 mL per tube with the help of automatic collector of BSZ-100 (Shanghai Jia Peng Technology Co., Ltd., Shanghai, China, No. 1205027). The elution fractions obtained were detected by reverse HPLC, and characterized by liquid chromatography–electrospray ionization–mass spectrometry (LC–ESI/MS) in a negative mode (see below). The tubes with relatively high content of STG were combined, concentrated by rotary evaporation to remove solvent under a certain reduced pressure, and lyophilized (Alpha 1-2 LD plus Lyophilizer: Marin Christ Company, Osterode, Germany, No. 21298) at −40 °C and 50 Pa, providing relatively high purity of STG.

Determination and Identification of Sesaminol Triglucoside and Its Hydrolysis Products

The determination STG and its hydrolysis products was carried out with a Waters 1525 series HPLC system (Waters, Milford, MA, USA), equipped with a UV detector at 290 nm and a C18 (4.6 mm × 150 mm × 5 μm) column from Phenomenex (Torrance, CA, USA) at 30 °C. The eluents of (A) methanol and (B) distilled water were used in the following conditions: 0–2 min (30 % A), 2–45 min (60 % A), and 45–58 (60 % A), and the flow rate was 1.0 mL/min. The quantification of STG as well as its hydrolysis products was calculated on the basis of the peak areas against the naringenin (internal standard) [8]. The samples taken out from the reaction mixture were centrifuged at 11,944×*g* for 5 min, and then filtered through a 0.45 μm microporous membrane (Teflon) to get rid of insoluble matters before HPLC analysis.

Identification of STG and its hydrolysis products produced in the process of enzyme reaction was achieved by LC–ESI/MS analysis [7]. LC–ESI/MS analysis was performed by using WATERS ACQUITY UPLC mass spectrometer (Waters, Milford, MA, USA) with a CSH C18 column (2.1 mm × 100 mm × 1.7 μm) and ESI in negative

mode. The eluents used were (A) acetonitrile and (B) 0.1 % formic acid. The elution conditions were 0–6 min (5 % A) and 6–8 min (60 % A), and the flow rate was 0.3 mL/min. The main ESI ion source operating parameters were as follow: drying gas, N_2; flow rate of desolvation gas, 500 L/h; capillary pressure, 3.0 kV; cone pressure, 30 V; source block temperature, 100 °C; desolvation temperature, 400 °C; collision energy (eV), 6 V; detector pressure, 1,800 V and the scanning range was 100–1,500 *m/z*.

Preparation of Sesaminol from Sesaminol Triglucoside by β-Glucosidase and Cellulase Hydrolysis

A slight modification was performed according to the study of Jan *et al*. [15]. In short, 0.8 mL STG aqueous solution (1, 2, 4, 6, 10, 15 mg/mL) were incubated with different dosages of two enzymes (25, 50, 100, 200 μL) at various ratios of β-glucosidase and cellulose [0:100 (v/v), 20:80, 40:60, 50:50, 60:40, 80:20, 100:0] in a final volume of 1 mL. All enzymatic hydrolysis were, respectively, performed in 50 mM acetate buffer, pH (2.8, 3.8, 4.8, 5.8, 6.8), in an electrical thermostatic oscillation sink (Shanghai Heng Technology Co., Ltd., Shanghai, China) at a various temperatures (30, 40, 50, 60, 70 °C) for 0–48 h. After incubation, the reactions were stopped by heating at 100 °C for 3 min. The samples were mixed with an equal volume of methanol to dissolve hydrolysis products, and centrifuged at 11,944×*g* for 5 min, then filtered through 0.45 μm microporous membranes (Teflon). 20 μL samples were analyzed by HPLC using the internal standard (naringenin) [8]. The yield of sesaminol was calculated by the following formula:

$$\text{Yield of sesaminol (\%)} = (\text{the amount of sesminol obtained})/(\text{the initial amount of STG}) \times 100.$$

Statistical Analysis

All experiments were done in triplicate and data in this research are shown as the mean values with their standard deviations (means ± SD). Differences in the yield of sesaminol between all assays were checked by a one-way analysis of variance (ANOVA) test using SPSS 19.0 for Windows (SPSS Inc., Chicago, USA). The statistical significance was determined by *post hoc* comparison tests of means at the level of $P < 0.05$.

Results and Discussion

Preparation and Identification of Sesaminol Triglucoside

The yield of STG crude extract was 19.4 % based on the DSC used. Afterwards, STG with a purity of 86.5 % was obtained by polyamide chromatography column purification.

The compound F-1 was determined by HPLC (t_R = 22.3 min, Fig. 1a). Then the chemical structure identification of F-1 was carried out by ESI–MS (Fig. 1b). Fragment at *m/z* 855.2 was the $[M–H]^-$ ion (relative molecular mass 856.2), fragments at *m/z* 891.1 and 892.2 were for $[M+Cl^-]^-$ ion, fragment *m/z* 902.2 was $[M+HCOO^-]^-$ ion. The ions of *m/z* 693.1 and 369.1 indicated that F-1 missed one molecule of glucose residues ($C_6H_{10}O_5$, 162 Da) and three molecules of glucose residues (482 Da). Fragment at *m/z* 179.0 and 485.1 were from $[C_6H_{12}O_6–H]^-$ and the three glucose residues, respectively, suggesting that the structure of F-1 at least has three molecules of glucose residues. Thus, it can be deduced that the F-1 is STG.

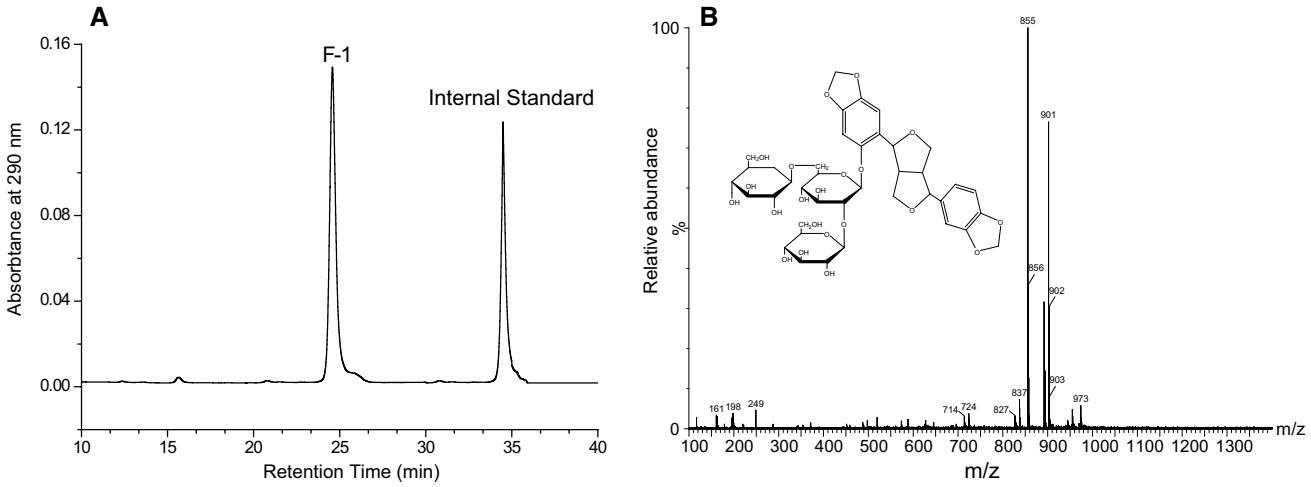

Fig. 1 **a** A typical reversed phase HPLC chromatogram of STG (t_R = 22.3 min); **b** ESI–MS spectrum of STG, *m/z* 855 $[M–H]^-$

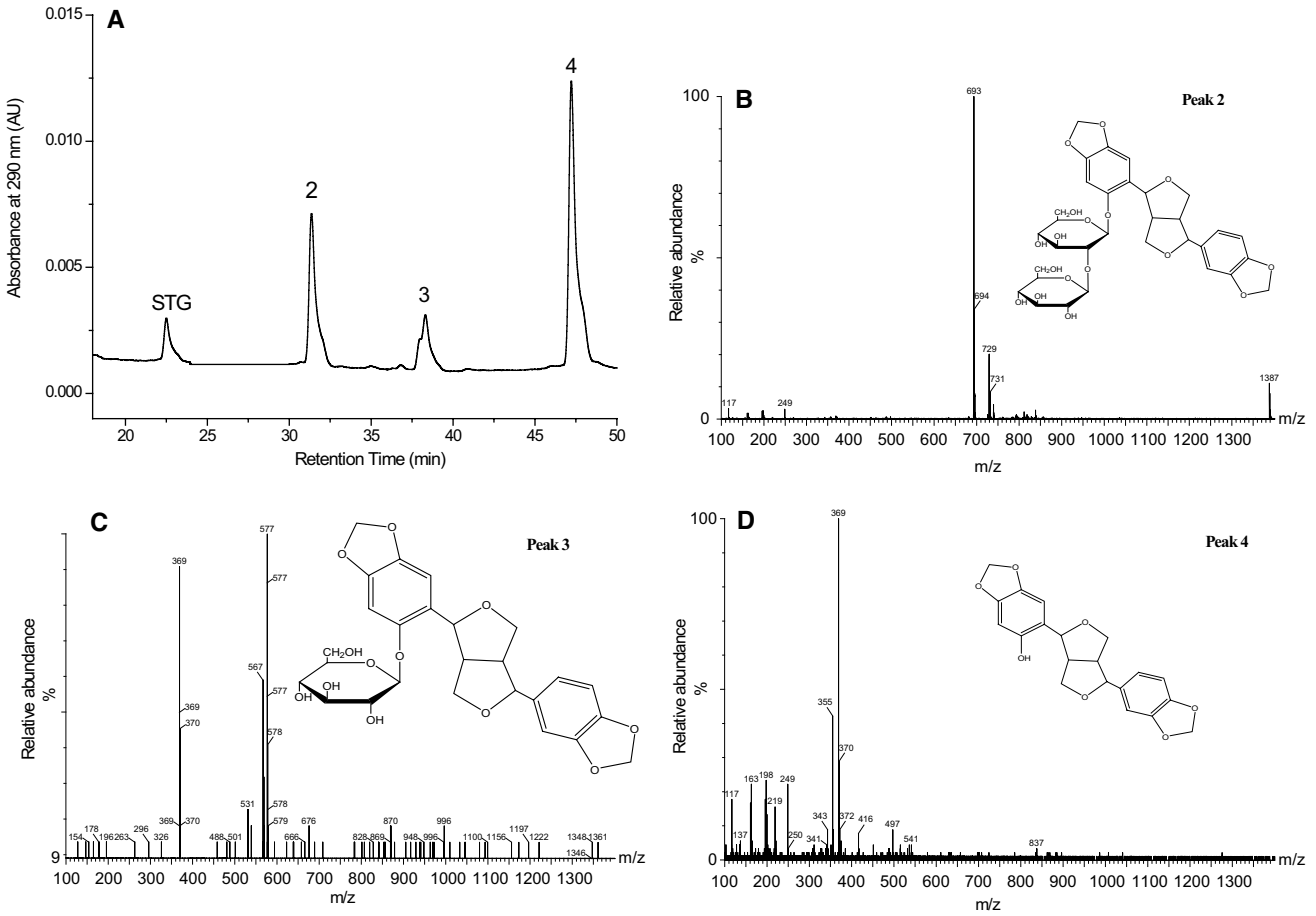

Fig. 2 **a** Reversed-phase HPLC analysis of STG and its hydrolysis products. *Peak 2*, SDG (t_R = 31.2 min); *peak 3*, SMG (t_R = 38.2 min); *peak 4*, sesaminol (t_R = 47.1 min); ESI–MS spectra of STG hydrolysis products: **b** *peak 2*, SDG, *m/z* 693 $[M–H]^-$; **c** *peak 3*, SMG, *m/z* 531 $[M–H]^-$; **d** *peak 4*, sesaminol, *m/z* 369 $[M–H]^-$

Determination and Identification of Sesaminol Triglucoside Hydrolysis Products

The process of the hydrolysis of STG catalyzed by β-glucosidase and cellulase at pH 4.8 and 50 °C was monitored by analytical reversed-phase HPLC (Fig. 2a). Sesaminol and other hydrolysis products (SDG and SMG) were identified by ESI–MS (Fig. 2). MS data (Table 1) was achieved from LC–ESI/MS analysis.

Typical retention times of peaks 2, 3, 4 resulting from the hydrolysis of STG (Fig. 1) under the standard HPLC conditions were as follows: peak 2, 31.2 min; peak 3, 38.2 min; peak 4, 47.1 min (Fig. 2). The LC–ESI/MS analysis of these hydrolysis products provided fragments $[M–H]^-$ at *m/z* 369 indicating that the compound 4 was sesaminol (Fig. 2d, relative molecular mass 370). Thus, we can concluded that sesaminol is the main hydrolysis products of STG due to the gradual hydrolysis with β-glucosidase and cellulase. The LC–ESI/MS analysis gave $[M–H]^-$ ions at *m/z* 693 and *m/z* 531 for peaks 2 and 3, which clearly suggested that these two products were SDG (Fig. 2b, relative molecular mass 694) and SMG (Fig. 2c, relative molecular mass 532), respectively.

Table 1 Sesaminol triglucoside and its hydrolysis products obtained by HPLC and LC–ESI/MS

Peak	Retention time (min)	ESI/MS *m/z* $[M–H]^-$	Assignment
STG	22.3	855	Sesaminol triglucoside
1	31.2	693	Sesaminol diglucoside
2	38.2	531	Sesaminol monoglucoside
3	47.1	369	Sesaminol

Retention time refers to HPLC analysis in Fig. 1 (STG, t_R = 22.3 min) and Fig. 2a (peak 2, SDG, t_R = 31.2 min; peak 3, SMG, t_R = 38.2 min; peak 4, sesaminol, t_R = 47.1 min)

Meanwhile, the changes of these compounds produced during the enzymatic hydrolysis are shown in Fig. 3. We found a gradual increase in sesaminol and glucose produced during the enzymatic hydrolysis of STG, with a transient yield of SDG at the early stage. Quite low amounts of SMG were detected during the enzymatic hydrolysis. ESI–MS analysis indicated that there were no dimers as well as trimers of glucoses in the enzymatic reaction. Sesaminol stereoisomers including 2-episesaminol, 6-episesaminol, as well as diasesaminol were not characterized during the enzymatic hydrolysis. After 6 h, sesaminol could be substantially produced from STG hydrolysis, and the maximum yield of sesaminol could be achieved at 12 h during hydrolysis. In contrast, a gradual increase of the SMG level was observed throughout the entire time course of reaction. These results suggested that sesaminol could be successfully prepared by the enzymatic hydrolysis of STG with β-glucosidase and cellulase, which was consistent with the previous studies [1, 20–22].

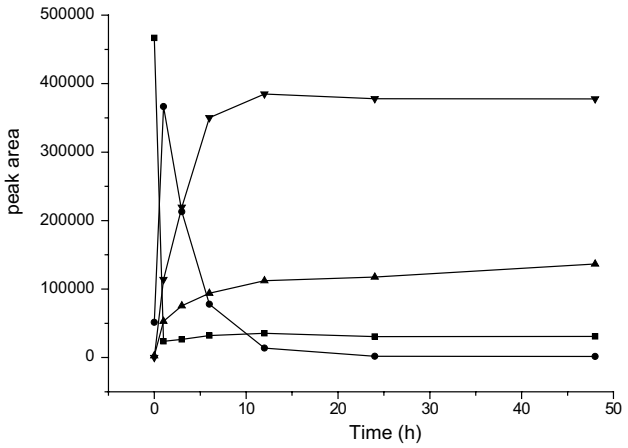

Fig. 3 Changes in STG and its hydrolysis products during hydrolysis with β-glucosidase and cellulase. 1.5 mL STG (8 mg/mL) was reacted with 200 μL β-glucosidase and cellulase (1:4, v/v) at pH 4.8 and 50 °C for 48 h. STG (*squares*), SDG (*circles*), SMG (*triangles up*), sesaminol (*triangles down*). *Each point* represents the mean ± SD ($n = 3$)

Effect of Total Enzyme Dosage on the Yield of Sesaminol

The yields of sesaminol determined by HPLC are shown in Fig. 4, in which the enzyme ratio of β-glucosidase and cellulase used was 1:4 (v/v). The reaction rate significantly increased ($P < 0.05$), reaching a maximum yield of sesaminol up to 28.2 % for an enzyme dosage of 200 μL, then decreasing significantly at a reaction time above 6 h ($P < 0.05$), indicating that sesaminol might readily undergo demethylenation, reduction or further oxidation into other products [23]. It was noticeable that the total enzyme dosage of 50 μL led to the highest yield of 16.8 % at the end of this enzymatic hydrolysis (48 h) which might be due to the lower transformation of sesaminol to its byproducts. This conversion was related to factors such as reaction time, the binding capacity of enzymes with substrate, sesaminol concentration, especially the dynamic synergistic interaction between β-glucosidase and cellulase [24]. The yields of 28.2 and 26.6 % of sesaminol were achieved at the enzyme dosages of 200 and 100 μL, respectively, which were significantly higher than those obtained from enzyme dosages of 50 and 25 μL ($P < 0.05$). However, no significant difference for sesaminol yields was observed for the total enzyme dosage of 200 and 100 μL ($P > 0.05$). Thus, the total enzyme dosage of 100 μL was selected considering the cost of enzyme and the yield efficiency.

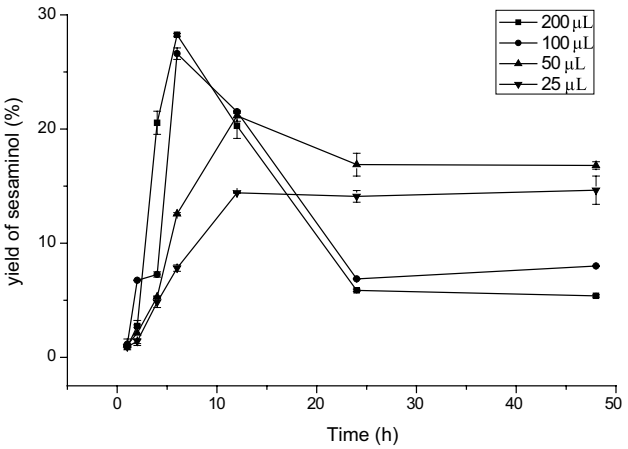

Fig. 4 Effect of total enzyme dosage on yield of sesaminol. The enzyme ratio of β-glucosidase and cellulase used was 1:4 (v/v). All reactions were carried out under the conditions of substrate concentration (0.8 mL, 6 mg/mL), pH 4.8, 50 °C after an appropriate time interval. *Each point* represents the mean ± SD ($n = 3$)

Effect of Ratio of Two Enzymes and Reaction Time on the Yield of Sesaminol

As shown in Fig. 5, relatively high yields of sesaminol were achieved with both β-glucosidase and cellulose. However, in the absence of β-glucosidase or cellulase, little sesaminol was obtained. For example, cellulase alone at 0:100 (v/v) or β-glucosidase alone at 100:0 released only 18.7 and 31.7 % of the total sesaminol from STG, respectively, but when β-glucosidase and cellulase were simultaneously added at a ratio of 60:40 and 20:80, the yields of sesaminol significantly increased to 43.5 and 41.3 % ($P < 0.05$), respectively.

These results might suggest a dynamic synergistic interaction between β-glucosidase and cellulase on hydrolysis of STG, which was in accordance with the findings of Kuriyama and Murui [25]. These authors showed that when

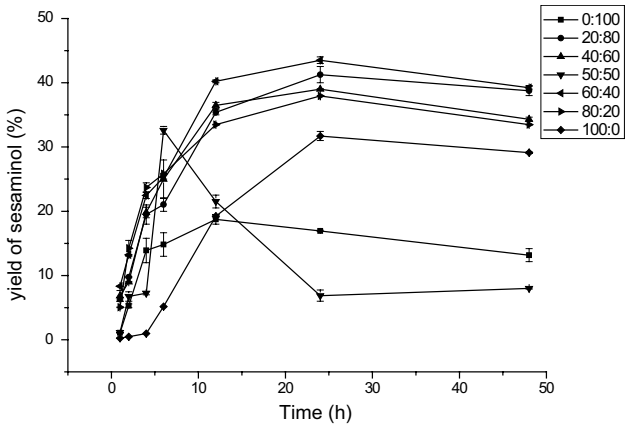

Fig. 5 Effect of the ratio of two enzymes and the reaction time on yield of sesaminol. During the reaction, the ratio of β-glucosidase and cellulase were 0:100 (v/v) (*squares*), 20:80 (*circles*), 40:60 (*triangles up*), 50:50 (*triangles down*); 60:40 (*triangles left*); 80:20 (*triangles right*); 100:0 (*diamond*). All reactions were carried out under the conditions of substrate concentration (0.8 mL, 6 mg/mL), pH 4.8, 50 °C after an appropriate time interval. *Each point* represents the mean ± SD ($n = 3$)

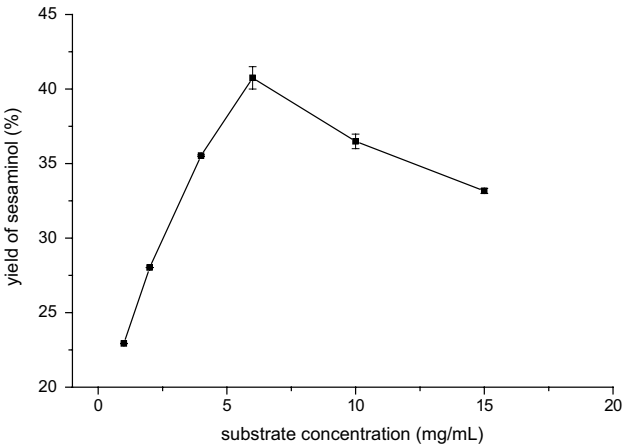

Fig. 6 Effect of substrate concentration (1, 2, 4, 6, 10, 15 mg/mL) on yield of sesaminol. The reaction was carried out under the conditions of β-glucosidase and cellulose (20:80, v/v) at pH 4.8, 50 °C after 24 h incubation. Data was expressed as the mean ± SD ($n = 3$)

cellulase was used with β-glucosidase, more abundant sesaminol was obtained by hydrolysis of STG. Based on these results, it could be deduced that two enzymes should be used together in order to efficiently prepare sesaminol from STG. The enzymatic hydrolysis was supposed to alter the physical and (or) chemical properties of STG or its intermediate products and made it easier for further enzymatic attack, since combination of enzymes and substrates might lead to the changes of glucose configuration in STG. However, the synergistic mode might vary according to the hydrolysis rate of STG by β-glucosidase and cellulase, the binding capacity of enzymes employed and substrate as well as the specificity of the two enzymes. There was no significant difference between the yields of sesaminol at the ratio of 60:40 and 20:80 ($P > 0.05$). Thus, the ratio of β-glucosidase and cellulase of 20:80 was chosen considering the relatively expensive cost of β-glucosidase compared with cellulase.

When the ratio of β-glucosidase and cellulase was 20:80 (v/v), the yield of sesaminol significantly increased up to 41.3 % ($P < 0.05$) at the initial stage. However, no significant increase was observed as the reaction time went beyond 24 h ($P > 0.05$). Therefore, the reaction time of 24 h was selected.

Effect of Substrate Concentration on the Yield of Sesaminol

Influence of substrate concentration (0.8 mL, 1–15 mg/mL) on the yield of sesaminol from STG by β-glucosidase and cellulose (20:80, v/v) at 50 °C after 24 h incubation is presented in Fig. 6. The relation between initial rates and substrate concentration followed a hyperbolic curve. Thus, lower substrate concentrations led to first order kinetics. It was shown that the reaction rate significantly increased ($P < 0.05$), reaching a maximum yield of sesaminol up to 40.8 % and then decreasing slightly following a substrate inhibition kinetics, at concentrations above 6 mg/mL of STG ($P > 0.05$). To the best of our knowledge, few researches are available to explain the affinity of the two enzymes for the substrate of STG. However, it seems that the two enzymes are capable of hydrolyzing the glycosidic bonds at a high hydrolysis rate in the beginning, and then a substrate inhibition effect might mainly influence the reaction rate of the two enzymes.

Therefore, the substrate concentration of 6 mg/mL was chosen for the optimization.

Effect of Reaction Temperature and pH on the Yield of Sesaminol

The maximum yield of sesaminol obtained was 40.8 % by both β-glycosidase and cellulase (20:80, v/v) at 50 °C (Fig. 7a) and a pH value of 4.8 (Fig. 7b), which could be justified with the fact that β-glycosidase and cellulase display the highest activity at pH 4.0 (37 °C) and pH 5.0 (55 °C), respectively. This also suggested that enzymes activity was sensitive to temperature and pH values. Too high or too low temperatures and pH values made the enzymes inactive, thereby led to the decline in the hydrolytic reaction rate. Therefore, the optimum temperature and pH was 50 °C, pH 4.8.

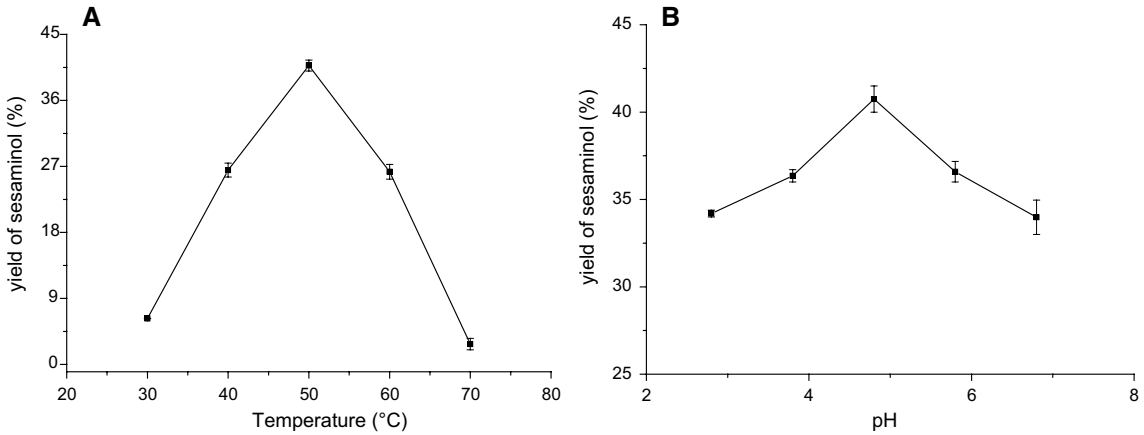

Fig. 7 **a** Effect of reaction temperature (30, 40, 50, 60, 70 °C) on yield of sesaminol. The reaction was carried out under the conditions of substrate concentration (0.8 mL, 6 mg/mL), β-glucosidase and cellulose (20:80, v/v) at pH 4.8 after 24 h incubation. **b** Effect of reaction system pH (2.8, 3.8, 4.8, 5.8, 6.8) on yield of sesaminol. The reaction was carried out under the conditions of substrate concentration (0.8 mL, 6 mg/mL), β-glucosidase and cellulose (20:80, v/v) at 50 °C after 24 h incubation. Data are expressed as means ± SD ($n = 3$)

Conclusion

In conclusion, a 19.4 % yield of STG crude extract based on the amounts of DSC used was successfully achieved. Moreover, relatively high-purity of STG (86.5 %) was obtained with polyamide column chromatography purification procedures. In addition, this study also demonstrated that sesaminol can be prepared from STG by β-glucosidase and cellulase hydrolysis. The optimal conditions for preparation of sesaminol (final volume 1 mL) were total enzyme dosage 100 μL (8,000.72 U), the ratio of β-glucosidase and cellulase 20:80 (v/v), reaction time 24 h, substrate concentration 6 mg/mL, reaction temperature 50 °C, and reaction system pH 4.8. Under these conditions, the experimental yield of sesaminol was 48.9 %. STG (*m/z* 855 $[M–H]^-$) and its hydrolysis products (SDG *m/z* 693 $[M–H]^-$, SMG *m/z* 531 $[M–H]^-$ and sesaminol *m/z* 369 $[M–H]^-$) were also successfully identified by LC–ESI/MS. This study provided a possibility for the large-scale enzymatic preparation of sesaminol from STG and the production of functional foods rich in sesaminol.

References

1. Park SH, Ryu SN, Bu Y, Kim H, Simon JE, Kim KS (2010) Antioxidant components as potential neuroprotective agents in sesame (*Sesamum indicum* L.). Food Rev Int 26:103–121
2. Ryu SN, Ho CT, Osawa T (1998) High performance liquid chromatographic determination of antioxidant lignan glycosides in some varieties of sesame. J Food Lipids 5:17–28
3. Namiki M (2007) Nutraceutical functions of sesame: a review. Crit Rev Food Sci Nutr 47:651–673
4. Kamal-Eldin A, Moazzami A, Washi S (2011) Sesame seed lignans: potent physiological modulators and possible ingredients in functional foods & nutraceuticals. Recent Pat Food Nutr Agric 3:17–29
5. Katsuzaki H, Kawakishi S, Osawa T (1933) Structure of novel antioxidative lignan triglucoside isolated from sesame seed. Heterocycles 36:933–936
6. Sarkis JR, Michel I, Tessaro IC, Marczak LDF (2014) Optimization of phenolics extraction from sesame seed cake. Sep Purif Technol 122:506–514
7. Zhu XL, Zhang X, Sun YK, Su D, Sun Y, Hu B, Zeng XX (2013) Purification and fermentation *in vitro* of sesaminol triglucoside from sesame cake by human intestinal microbiota. J Agric Food Chem 61:1868–1877
8. Moazzami AA, Andersson RE, Kamal-Eldin A (2006) HPLC analysis of sesaminol glucosides in sesame seeds. J Agric Food Chem 54:633–638
9. Hemalatha S, Ghafoorunissa (2004) Lignans and tocopherols in Indian sesame cultivars. J Am Oil Chem Soc 81:467–470
10. Miyake Y, Fukumoto S, Okada M, Sakaida K, Nakamura Y, Osawa T (2005) Antioxidative catechol lignans converted from sesamin and sesaminol triglucoside by culturing with Aspergillus. J Agric Food Chem 53:22–27
11. Suja KP, Jayalekshmy A, Arumughan C (2004) Free radical scavenging behavior of antioxidant compounds of sesame (*Sesamum indicum* L.) in DPPH center dot system. J Agric Food Chem 52:912–915
12. Katsuzaki H, Kawakishi S, Osawa T (1994) Sesaminol glucosides in sesame seeds. Phytochemistry 35:773–776
13. Miyahara Y, Hibasami H, Katsuzaki H, Imai K, Osawa T, Ina K, Komiya T (2001) Sesaminol from sesame seed induces apoptosis in human lymphoid leukemia Molt 4B cells. Int J Mol Med 7:485–488
14. Huang J, Song GH, Zhang L, Sun Q, Lu X (2012) A novel conversion of sesamolin to sesaminol by acidic cation exchange resin. Eur J Lipid Sci Technol 114:842–848
15. Jan KC, Ku KL, Chu YH, Hwang LS, Ho CT (2011) Intestinal distribution and excretion of sesaminol and its tetrahydrofuranoid metabolites in rats. J Agric Food Chem 59:3078–3086
16. Fukuda Y, Osawa T, Namiki M, Ozaki T (1985) Studies on antioxidative substances in sesame seed. Agric Biol Chem 49:301–306

17. Nair A, Kuwahara A, Nagase A, Yamaguchi H, Yamazaki T, Hosoya M, Omura A, Kiyomoto K, Yamaguchi MA, Shimoyama T, Takahashi S, Nakayama T (2013) Purification, gene cloning, and biochemical characterization of a β-glucosidase capable of hydrolyzing sesaminol triglucoside from *Paenibacillus* sp. KB0549. PLoS One 8:e60538
18. AOAC (1995) Official methods of analysis, 16th edn. Association of Official Analytical Chemists, Washington, DC
19. Ghose TK (1987) Measurement of cellulase activities. Pure Appl Chem 59:257–268
20. Kang MH, Kawai Y, Naito M, Osawa T (1999) Dietary defatted sesame flour decreases susceptibility to oxidative stress in hypercholesterolemic rabbits. J Nutr 129:6
21. Tamura G, Gold C, Ferro-Luzzi A, Ames BN (1980) Fecalase: a model or activation of dietary glycosides to mutagens by intestinal flora. Proc Natl Acad Sci USA 77:4961–4965
22. Stahl W, van den Berg H, Arthur J, Bast A, Dainty J, Faulks RM, Gärtner C, Haenen G, Hollman P, Holst B, Kelly FJ, Polidori MC, Rice-Evans C, Southon S, van Vliet T, Viña-Ribes J, Williamson G, Astley SB (2002) Bioavailability and metabolism. Mol Asp Med 23:39–100
23. Jan KC, Hwang LS, Ho CT (2009) Biotransformation of sesaminol triglucoside to mammalian lignans by intestinal microbiota. J Agric Food Chem 57:6101–6106
24. Nga IS, Tsai SW, Jud YM (2011) Dynamic synergistic effect on *Trichoderma reesei* cellulases by novel β-glucosidases from Taiwanese fungi. Bioresour Technol 102:6073–6081
25. Kuriyama K, Murui T (1993) Effect of cellulase on hydrolysis of lignan glycosides in sesame seed by beta-glucosidase. Nippon Nogeikagaku Kaishi 67:1701–1705