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Biotransformation of Gingerol-Related Compounds in Ginger Rhizome Extract with Selected Species of Aspergillus, Monacus and Penicillium

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ABSTRACT

The simultaneous biotransformation of gingerol-related compounds in ginger extract was performed by *Aspergillus niger*, *A. flavas*, *Monacus purpureus* and *Penicilium funiculosum*. Ginger metabolites and their substrates from the biotransformation were characterized by HPLC and HPLC-QTOF-MS. GM-1, GM-2 and GM-3 were converted from [6]-gingerol and [6]-shogaol, the main constituents found in the ginger extract, by both strains of *A. niger* after a 24-h fermentation period. Meanwhile, *P. funiculosum* was able to produce GM-2 and GM-3. The enzymatic reactions of the substrate molecules included the reduction of ketone groups as well as hydroxylation of terminal alkyl side chains. The levels of almost all gingerol-related compounds in the cultivation medium of all fungi, were decreased. There was no metabolite derived from the other compounds in the homologous series of gingerols and shogaols according to the mentioned reactions. In conclusion, the present study verified that simultaneous biotransformation was an effective technique to produce a variety of active metabolites and the crude extract from natural products can be applied to biotransformation studies without any isolation or purification steps for each substrate.

Keywords: *Aspergillus niger*; Biotransformation; Fungal strains; [6]-gingerol; Ginger extract; Mass spectrometry; [6]-shogaol; *Zingiber officinale* Roscoe

1. Introduction

Ginger (*Zingiber officinale* Roscoe) rhizome is used as a spice for various foods and beverages in many cuisines. It is also an important component in traditional herbal medicines all over the world for the treatment of several diseases and symptoms including nausea and vomiting, indigestion,

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constipation, osteoarthritis and muscular pains [1]. Furthermore, ginger has many potential biological activities such as anti-inflammatory, anti-oxidative, anti-cancer and anti-microbial effects [1, 2]. The constituents of this plant are numerous and vary depending on where it was grown and whether the rhizome is fresh or dry. The biologically active compounds of ginger can be classified into two main categories, i.e., volatile terpenes and non-volatile phenolic compounds [3]. Terpene components include zingiberene, β-bisabolene, α-farnesene, α-curcumene and β-sesquiphellandrene which are the main constituents of essential oils providing the aroma and taste of ginger. Phenolic compounds are mainly gingerols contributing to the characteristic pungency of ginger [4, 5]. Gingerol-related compounds can be generally divided into gingerols, shogaols, paradols, gingerdiones and gingerdiols (Fig. 1). All of them contain the 3-methoxy-4-hydroxyphenyl moiety, except for methyl gingerols which have 3,4-dimethoxyphenyl. Each group is considered as a homologous series with varying length of unbranched alkyl chains [6]. [6]-Gingerol $(n=4)$ is the predominant compound in fresh rhizomes, while other gingerols such as [4]-, [8]-, [10]- and [12]-gingerol (n = 2, 6, 8 and 10, respectively) are present in lower concentrations [7].

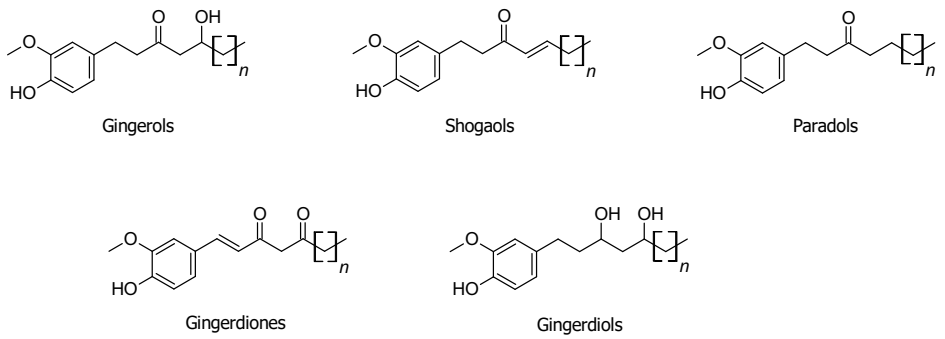


Fig. 1. Types of gingerol-related compounds found in ginger (n = number of carbon atoms).

During the preparations of either dried ginger powder or ginger extract using high temperature, some gingerols are converted to their corresponding shogaols, zingerone, and other related compounds, of which [6]-shogaol $(n=4)$ is the most prevalent product [7]. Various extraction methods have successfully obtained bioactive compounds from ginger and the most interesting compounds in ginger include [6]-gingerol and [6]-shogaol. These two compounds display substantially different biological activities, although both share similar chemical groups. In many cases, [6]-shogaol has been reported to have better pharmacological activities than [6]-gingerol [7]. Altered chemical profiles of ginger extract, achieved through modifying the extraction process, can improve the anti-cancer activity of said extracts [8]. The structural features of gingerol-related compounds contribute significantly to the enhancement of biological activities [1]. Therefore, the structural modification of these compounds may produce novel derivatives, and biotransformation could be an effective method for this purpose.

Biotransformation is the process by which microorganisms change a specific substrate into a structurally similar product. Many benefits can be obtained from the biotransformation of natural products [9, 10]. Several publications have presented the biotransformation of major components from ginger; however, these publications have focused only on *Aspergillus niger* [11-14]. As seen in the literature, researchers have commonly used pure forms of the active compounds in ginger. [6]-gingerol [11], [6]-

shogaol [11-13] and [6]-dehydroparadol [14] have been converted to many metabolites, and their metabolic pathways have been shown.

In this study, crude ginger extract containing a mixture of gingerol-related compounds was studied for use as substrates for the fungal biotransformation. The ginger metabolite profiling resulting from the biotransformation was characterized based on HPLC-QTOF-MS analysis. The effect of different fungal strains on the production of metabolites was compared.

2. Materials and Methods

2.1 Chemicals

The reference standards of [6]-gingerol and [6]-shogaol (purity ≥ 98% by HPLC) were obtained from FortopChem Technology Limited (Hong Kong, China). Potato dextrose agar (PDA) and potato dextrose broth (PDB) from HiMedia Laboratories (Mumbai, India) were used for the cultivation of fungi. All solvents used for extraction and HPLC analysis were AR grade, purchased from RCI Labscan (Bangkok, Thailand), while all solvents used for HPLC-QTOF-MS analysis were LC-MS grade, purchased from Fischer Chemical (Leicestershire, UK).

2.2 Microorganism and culture conditions

The standard fungal strains were *A. niger* TISTR 3152, *A. niger* TISTR 3240, *A. flavas* TISTR 3041, *Monacus purpureus* TISTR 3385, and *Penicilium funiculosum* TISTR 3563 obtained from Thailand Institute of Scientific and Technological Research (TISTR, Pathum Thani, Thailand). The fungal cells kept in the form of revival freeze-dried culture were reactivated in PDB for 2 days and subsequently grown on PBA for 5 days in an incubator (Memmert, Schwabach, Germany) at 30°C. In each experiment, the 1-week refreshed fungal strain was maintained on agar and subcultured into the primary seed culture medium. The seed culture was prepared in a 250-mL Erlenmeyer flask containing 100 mL of PDB, which was kept on an orbital shaker (Revco Scientific, Asheville, NC) at 120 rpm for 24 h, at room temperature. All aseptic techniques were conducted in the biosafety level 2 laboratory containing a class II biological safety cabinet (Forma Scientific Inc., Marietta, OH).

2.3 Ginger extract

Rhizomes of ginger (*Z. officinale* Roscoe) were collected from Phayao province, Thailand in January 2005. Fresh plants were sliced into small pieces, dried in the hot sun for 5 days and then ground into powder. The dried ginger powder was kept in a dark, cool and dry place for 14 years. Each 50 g of ginger powder was extracted by Soxhlet apparatus using 300-mL methanol for 6 h and then filtered. The filtrate was evaporated for drying under reduced pressure at 55°C. The ginger extract was kept in an aluminum foil package and stored in a desiccator until further use. For determination of [6]-gingerol and [6]-shogaol content, 500 mg of ginger extract was dissolved in 5.0 mL of methanol, then used to make a 10-fold dilution for HPLC analysis.

2.4 Biotransformation of ginger extract

A 10.0 mL seed culture of each fungal strain was added to 90.0 mL of fresh PDB in a 250-mL Erlenmeyer flask and incubated on an orbital shaker. After 24 h, 500 mg of ginger extract dissolved with 1.0 ml of 30% ethanol was added to the culture medium. The fermentation conditions were maintained as described above. The total biotransformation media collected at 0, 1, 2, 4, 6 and 24 h were analyzed.

2.5 Sample preparation

At the end of each cultivation, total biotransformation medium was separated from the mycelia by filtration through cotton wool. A 50.0-mL clear broth was extracted with ethyl acetate (1:1 by volume) by vigorously mixing for 10 min in a separating funnel. After a two-phase separation, 25.0 mL of the organic layer was evaporated, and a dried residue was then adjusted to 2.0 mL with methanol. The sample was filtered through a 0.45 μm

VertiClean nylon syringe filter (Vertical Chromatography, Bangkok, Thailand) and kept in -20°C before analysis.

2.6 HPLC-UV analysis

Samples (5 µl) were determined by an HPLC system (Shimadzu, Kyoto, Japan) which consisted of a solvent delivery system (LC-10ATVP), a system controller (CBM-20A), an autosampler (SIL-20ACHT), an oven (CTO-10ASVP) and a UV-Vis detector (SPD-10AVP). The chromatographic separations were carried out on Kinetex® C18 columns (250±4.6 mm i.d.; 4 µm) fitted with a guard cartridge packed with C18 material (Phenomenex, Torrance, CA). Temperature was maintained at 30°C. A mixture of methanol and water (70:30) was used as the mobile phase, at a flow rate of 1.2 mL/min. The UV detector was set at a wavelength of 282 nm. The contents of [6]-gingerol and [6]-shogaol were estimated from their concentrations in the culture medium, calculated using the calibration curves of authentic standards. The standard solutions were prepared at concentrations ranging from 10-1,000 µg/mL; they were stored at -20°C until used.

2.7 HPLC-QTOF-MS analysis

The biotransformation samples were consecutively analyzed by HPLC-QTOF-MS (Agilent Technologies, Singapore) system which consisted of an HPLC system (Agilent 1260 Infinity series), a QTOF mass spectrometer (Agilent 6540 UHD) and an electrospray ionization (ESI) interface. The chromatographic separation was achieved on a Poroshell 120 EC-C18 (50±3.0 mm i.d.; 2.7 µm; Agilent) with the temperature maintained at 35°C. The mobile phases used were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient program for the HPLC was as follows: 0-17 min, 40-100% B; 17-21 min, 100% B; 21-21.10 min, 100-40% B; and 21.10-29 min, 40% B. The flow rate was set at 0.2 mL/min and the sample volume injected was 5 µL. The ion source was set in positive detection mode and the MS parameters were gas temperature 350°C; gas flow 10 L/min; nebulizer 30 psig, capillary 3500 V, fragmentor 100 V skimmer 165 V; Octopole RF Peak 750 V; mass range 50-800 *m/z*; scan rate 4.00 spectra/sec). All acquisition data were analyzed by the MassHunter software (Agilent Technologies, Santa Clara, CA).

3. Results and Discussion

3.1 Contents of [6]-gingerol and [6]-shogaol

The extraction of dried ginger powder with methanol resulted in a 14.95±0.40% yield of crude extract. In the present study, the contents of [6]-gingerol and [6]-shogaol in the ginger extract analyzed by HPLC were 7.67±0.28 and 12.27±0.68 mg/g, respectively. [6]-shogaol, the main degradation product of [6]-gingerol, was shown to be a major component in the ginger extract, present at a level approximately 1.5-fold higher than [6]-gingerol. These results imply that the drying process of fresh ginger, the long-term storage of ginger powder, and the extraction of ginger powder with high temperatures directly affect the conversion of [6]-gingerol to [6]-shogaol [3, 7, 15, 16].

3.2 Effect of varying fungal strains on the biotransformation of ginger extract

The disadvantage of pure substances extracted from ginger is that they are often more expensive and unavailable. In contrast, ginger extract can be produced locally at a lower cost and high quantity. Thus, it can be used as an alternative for biotransformation studies. To investigate the profiles of major substrates resulting from biotransformation, the crude ginger extract containing high levels of [6]-gingerol and [6]-shogaol was incubated in various culture media, each seeded with an individual strain of fungi for 24 h. Each total medium sample was quantitatively analyzed by HPLC. The control experiment without any fungal strains showed no alteration of substrate concentrations in the medium throughout the incubation; the resulting average

concentrations of [6]-gingerol and [6]-shogaol were 490.81 and 522.75 µg/mL, respectively. As shown in Fig. 2, no differences were found between the five strains of selected fungi. The contents of both active ingredients declined rapidly within 1 h after adding the substrate; after that, concentrations were roughly constant until 24 h. Additionally, the remaining levels of [6]-gingerol ranged from 46 to 58%, while low amounts of [6]-shogaol were observed in the medium. The reduction in the amount of both compounds resulted from metabolization by the fungi. The procedures for isolation and identification of metabolites generally required a large number of samples. These were time-consuming processes and these metabolites cannot be determined by HPLC-UV. Therefore, the characterization of ginger metabolites was accomplished by HPLC-QTOF-MS.

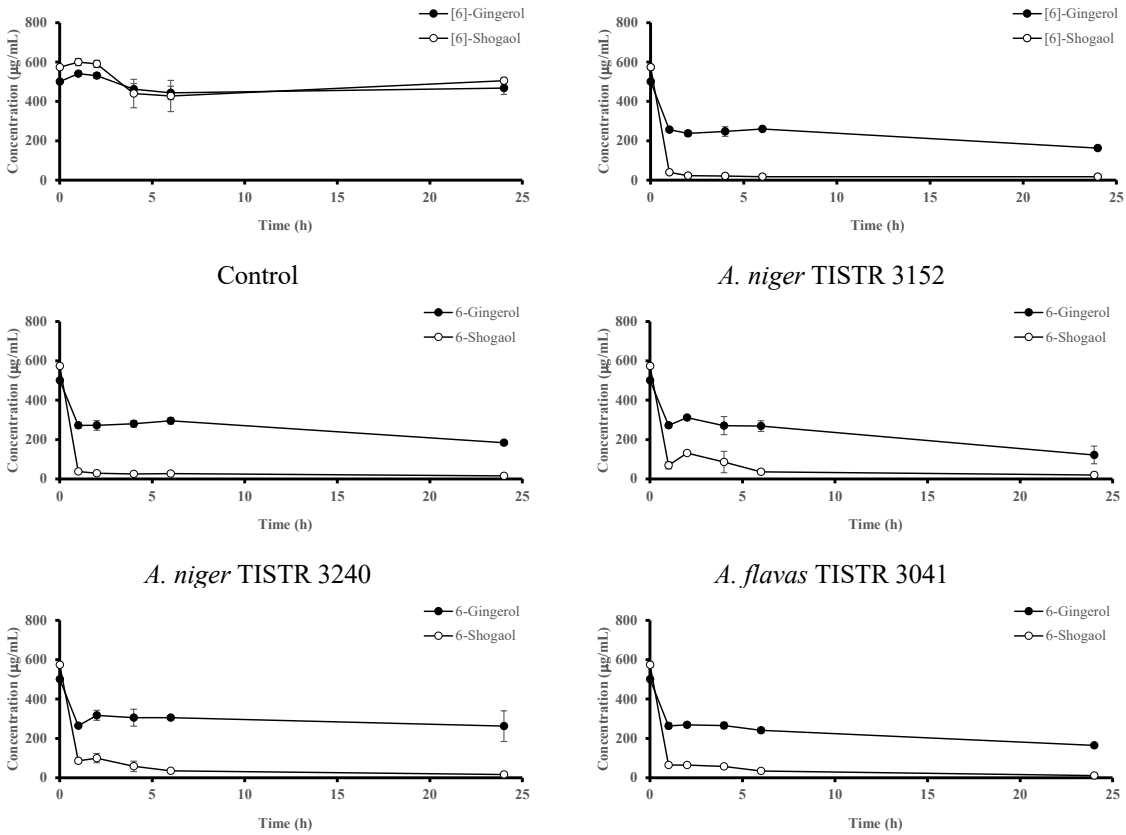


Fig. 2. Effect of different fungal strains on the concentration of [6]-gingerol and [6]-shogaol analyzed by HPLC-UV (n = 3).

3.3 Simultaneous biotransformation of [6]-gingerol and [6]-shogaol in ginger extract

The selected biotransformation samples with various fungal strains were analyzed by HPLC-QTOF-MS in a full-scan, single MS mode with positive ion detection to obtain the total ion chromatograms (TIC). Fig. 3 shows TIC and the corresponding extracted ion chromatogram (EIC) and mass spectrum (MS) for the control sample without any fungus at 1-h incubation. The peaks of [6]-gingerol and [6]-shogaol were detected using product ions according to their fragmentation patterns (Fig. 4). From the MS of two major peaks from the biotransformation samples, it was found that the peak of [6]-gingerol at 6.4 min showed a high intensity of $[M+Na]^+$ at *m/z* 317 followed by $M+H-H_2O]^+$ at *m/z* 277, but a very low intensity of $[M+H]^+$. However, the peak of [6]-shogaol at 10.3 min showed a high intensity of both $[M+Na]^+$ and $[M+H]^+$ at *m/z* 299 and 277, respectively, but a very low intensity of $[M+H-H_2O]^+$. These major product ions of each substrate are in agreement with previous reports [17, 18]. According to the EIC at their selected *m/z*, the alteration of contents for [6]-gingerol and [6]-shogaol in the biotransformation samples represented as peak heights are shown in Fig. 5. Although [6]-shogaol was higher in concentration than [6]-gingerol in the control experiment when analyzed by HPLC, the peak height of [6]-shogaol at *m/z* 299 was less than that of [6]-gingerol at *m/z* 317. This could have occurred due to the sharing of MS responses between both $[M+Na]^+$ and $[M+H]^+$ fragment ions of [6]-shogaol. Of all the fungal strains screened, the decline of [6]-shogaol levels at 1 and 24-h of fermentation was more than those of [6]-gingerol. These results correspond with the previous HPLC analysis. It should be noted that both substrates transformed into their respective metabolites.

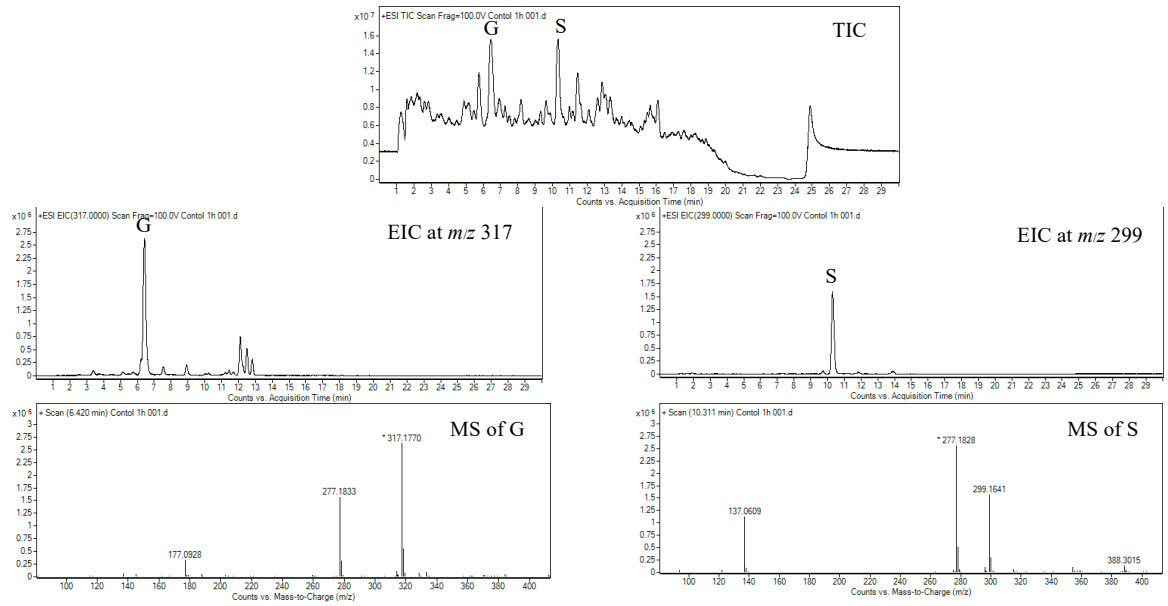


Fig. 3. Total ion chromatogram, EIC and MS of [6]-gingerol (G) and [6]-shogaol (S) for control experiment without any fungus at 1-h incubation.

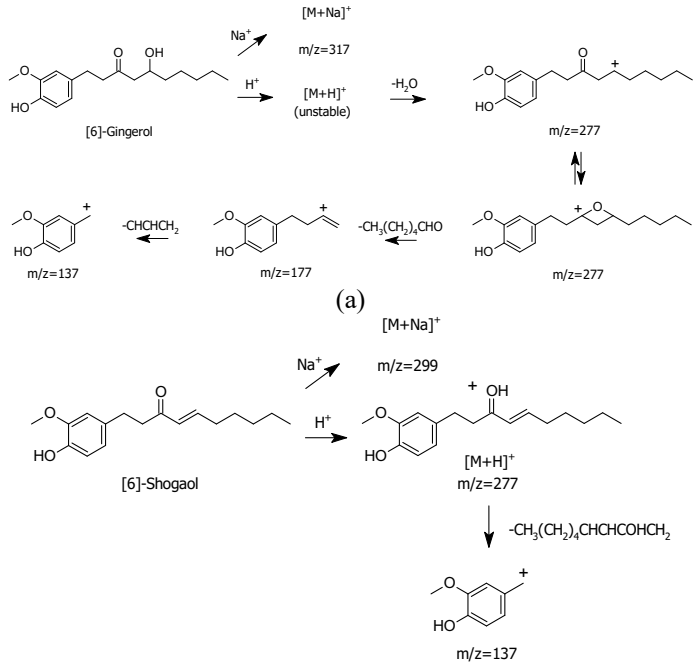


Fig. 4. (+)ESI-MS fragmentation pattern of [6]-gingerol (a) and [6]-shogaol (b).

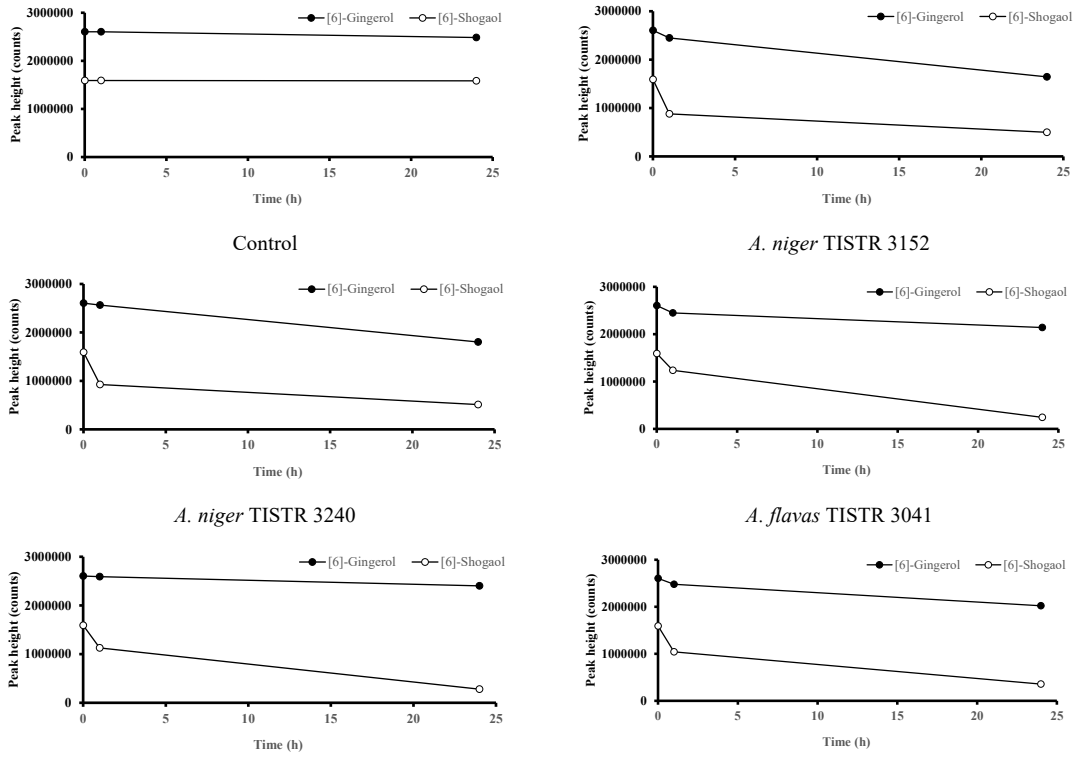


Fig. 5. The peak height of extract ion chromatogram of [6]-gingerol and [6]-shogaol in biotransformation samples with different fungal strains at 0, 1 and 24 h of incubation with different fungal strains (n = 1).

3.4 Characterization of ginger metabolites in the biotransformation

Several published papers have reported on the biotransformation of [6]-gingerol and [6]-shogaol using *A. niger* [11-14]. According to these reports, both compounds were converted to their metabolites in the more polar forms via chemical reactions such as reduction of the carbonyl group, hydroxylation of the alkyl side chain, and reduction of the carbon-carbon double bond. Based on the MS fragmentation patterns of metabolites reported by Takahashi et al. [11] and Jo et al. [13], together with our HPLC-QTOF-MS data, the ginger metabolites (GM) of [6]-gingerol and [6]-shogaol resulting from the biotransformation, have been tentatively identified. We have found three GMs after adding the ginger extract into the fermentation. The scheme for biotransformation pathways is presented in Fig. 6. The base peaks of GM-1, GM-2 and GM-3 were observed in EIC at *m/z* 279, 317 and 333, respectively, and the peaks were found in the chromatogram at 2.9, 3.8 and 1.9 min, respectively (Fig. 7). All more polar metabolites had faster elution times than [6]-gingerol (6.4 min) and [6]-shogaol (10.3 min). GM-2 and [6]-gingerol have the same *m/z* but they had different retention times.

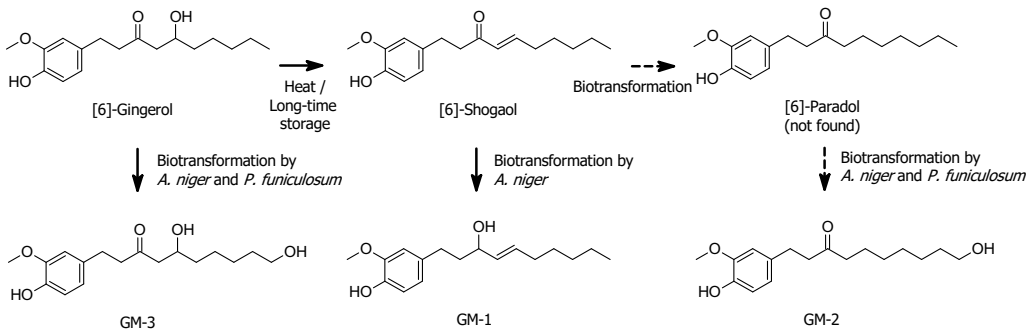
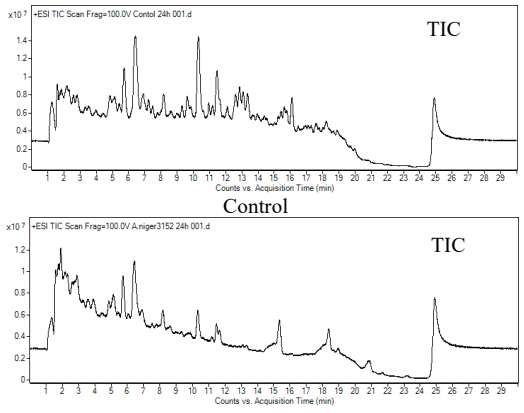
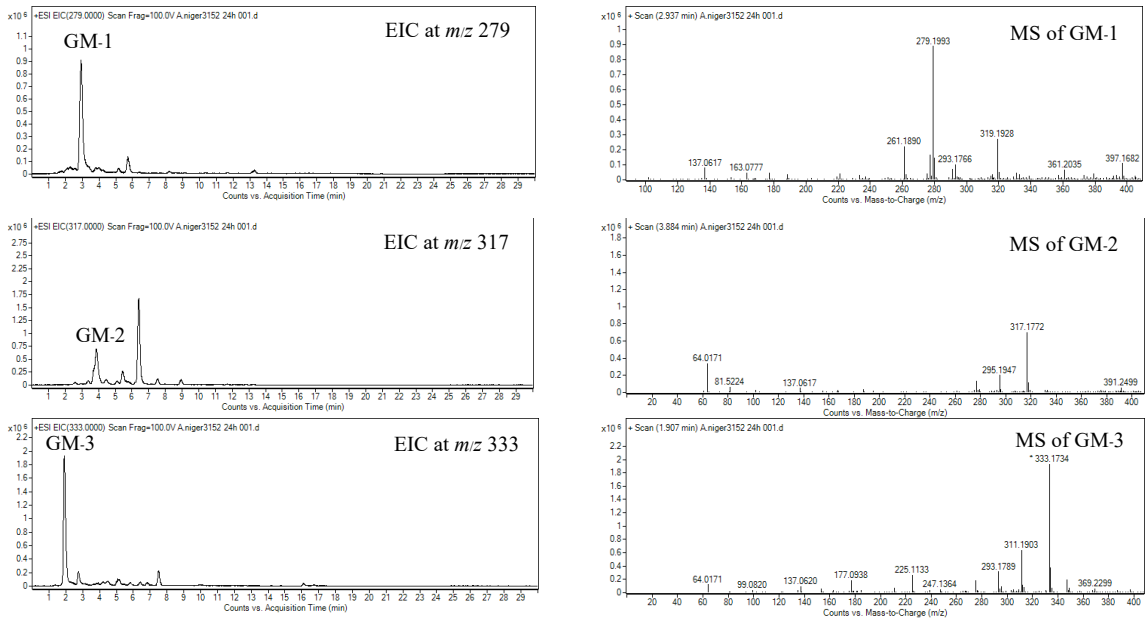


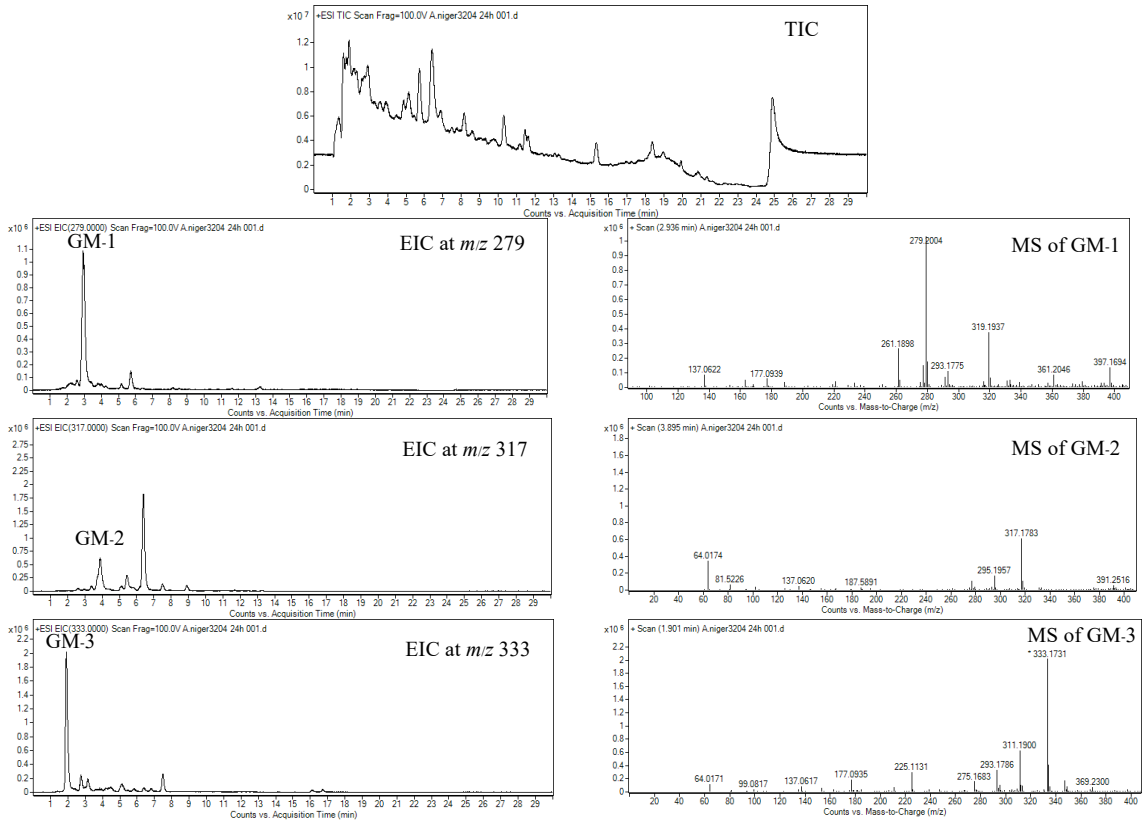
Fig. 6. Biotransformation pathway of the ginger extract containing [6]-gingerol and [6]-shogaol to GM-1 (1-(4′-hydroxy-3′-methoxyphenyl)-4-decen-3-ol), GM-2 (1-hydroxy-[6]-paradol), and GM-3 (1-hydroxy-[6]-gingerol) via actual step (solid line arrow) and predicted pathways (dashed line arrow).



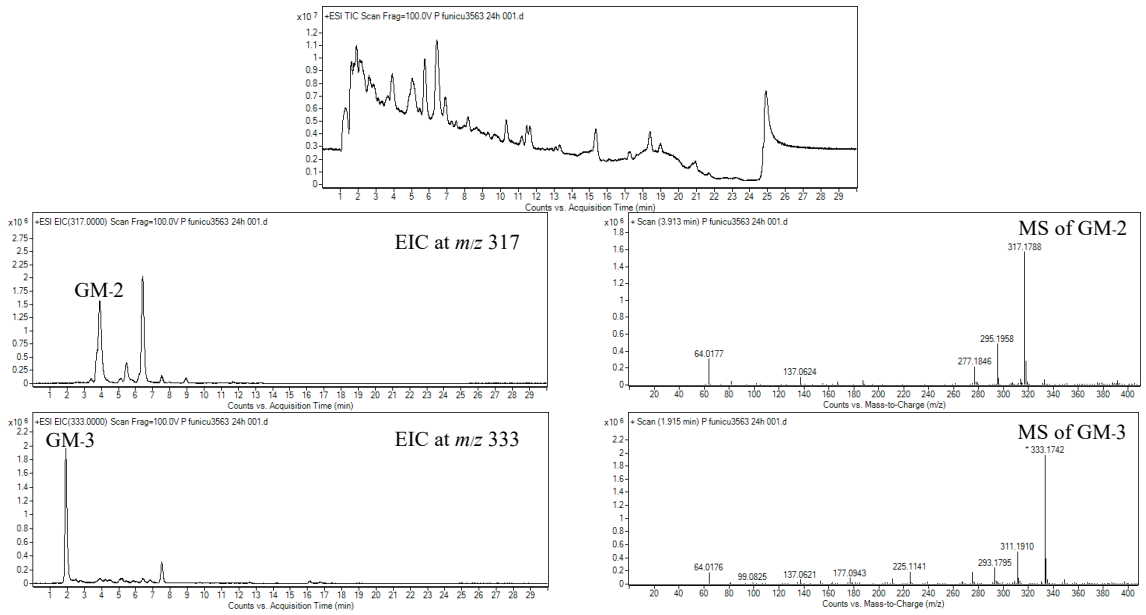


A. niger TISTR 3152

Fig. 7. TIC, EIC and MS of three GMs for the biotransformation of ginger extract with different fungal strains at 24-h fermentation.



A. niger TISTR 3240



P. funiculosum TISTR 3563

Fig. 7. TIC, EIC and MS of three GMs for the biotransformation of ginger extract with different fungal strains at 24-h fermentation (cont.).

The fragment ions from the GMs are shown in Fig. 8. The GMs and their substrates including other gingerol-related compounds showed a characteristic fragment ion at *m/z* 137 ($[C_8H_9O_2]^+$) by cleavage of the C-C bond between the aromatic ring and the carbonyl moiety. GM-1 showed the element composition of $C_{17}H_{26}O_3$ and its product ion at *m/z* 261 was generated by the loss of H_2O from *m/z* 279 ($[M+H]^+$), indicating the presence of a hydroxyl group in the precursor ion. Therefore, GM-1 was identified as 1-(4′-hydroxy-3′-methoxyphenyl)-4-decen-3-ol degrading from the oxidation of [6]-shogaol. GM-2 ($C_{17}H_{26}O_4$) and GM-3 ($C_{17}H_{26}O_5$) were detected as sodium adduct ions (*m/z* 317 and 333, respectively), while the *m/z* 295 and 311 corresponded to their protonated molecular ions, together with the *m/z* 277 and 293 matched for H_2O subtracted protonated molecular ions, respectively. The MS fragmentation pattern of GM-2 was related to 1-hydroxy-[6]-paradol which was possibly degraded from [6]-paradol. Indeed, [6]-paradol is the intermediate metabolite between the pathway for the transformation of [6]-shogaol to GM-2, suggested by Lee [12] and Jo et al. [13] However, the present study did not find [6]-paradol in either control or fermented medium. The hydroxylation at ω-1 and ω-2 of [6]-gingerol was caused by *A. niger* [9], but GM-3 should be identified as 1-hydroxy-[6]-gingerol rather than 2-hydroxy-[6]-gingerol because Takahashi et al. [11] confirmed that the primary alcohol is a major metabolite that can be further degraded to other metabolites.

In the presence of *A. niger* TISTR 3152 and *A niger* TISTR 3240, [6]-shogaol was converted to GM-1 and GM-2, but [6]-gingerol was transformed to GM-3. *A. niger* can produce different kinds of enzymes and thus is able to degrade a large

array of organic compounds [19]. Besides this, *P. funiculosum* TISTR 3563 gave two metabolites, GM-2 and GM-3, which had not been previously reported. All of the compounds were previously known metabolites. On the other hand, no metabolites were found from *A. flavas* TISTR 3041 and *M. purpureus* TISTR 3385. The content of [6]-gingerol substrate was lower than [6]-shogaol in the ginger extract, but GM-3 was predicted as the main metabolite in three different fungal strains based on the peak height of EIC. These findings are consistent with those reported by Takahashi et al. [11], demonstrating that GM-3 was the major metabolite produced by *A. niger* using [6]-gingerol as a substrate. Our results concurred with Jo et al. [13] since the α,β-unsaturated ketone of [6]-shogaol was easily reduced to the corresponding allylic alcohol (GM-1) as an important product**.** Meanwhile, Takahashi et al. [11] suggested that fermentation of [6]-shogaol did not result in GM-1 in the medium, though they found two main products with ω1-hydroxylation, keto alcohol (GM-2) and diol metabolites, after a 2-day cultivation. Overall, the profiles of GMs may depend on the incubation period and/or the fungal strains used.

With regards to the alteration of peak heights in the biotransformation samples (Fig. 9), the increase of both GM-1 and GM-2 correlated to the decline of [6]-shogaol in both *A. niger* fermentations. In the case of *P. funiculosum* TISTR 3563, the concentration of GM-2 was higher than those of both *A. niger* strains. This might be due to *P. funiculosum* TISTR 3563 preferentially converting [6]-shogaol to GM-2 only, but *A. niger* degraded [6]-shogaol to GM-1 and GM-2 by dual pathways. On the contrary, the amount of GM-3 rapidly increased while the amount of [6]-gingerol gradually decreased. Consequently, it should be the case that GM-3 is derived from gingerol-related compounds, other than [6]-gingerol, found in the ginger extract.

3.5 Examination of the gingerol-related compounds in the biotransformation

According to the MS data for identification of constituents in methanol ginger extract presented by Cheng et al. [8], Park and Jung [17], and Jiang et al. [20], we could determine the gingerol-related compounds i.e., gingerols, shogaols, paradols, gingerdiones and gingerdiols in the medium during the biotransformation. Table 1 shows the tentative identifications for the major compounds including [6]-gingerol and [6]-shogaol found in the control experiment with ginger extract. The results show that the sequence of retention times in reverse-phase HPLC, as well as the fragmentation behaviors for twelve compounds, were referenced in previous studies [8, 17, 20]. By comparing these data to the peak heights of EIC for these compounds, diacetoxy-[6]-gingerdiol was roughly estimated to be the most abundant component, followed by [6]-gingerol, [6]-shogaol, methyl diacetoxy-[6]-gingerdiol and then [6]-gingerdiol. Notably, we found that the contents of compounds with a side-chain length with $n = 4$ ([6]-) were significantly higher than those with $n = 2$ ([4]-), 6 ([8]-) and 8 ([10]-). However, many components were not detectable, as they might have been present at very low concentrations.

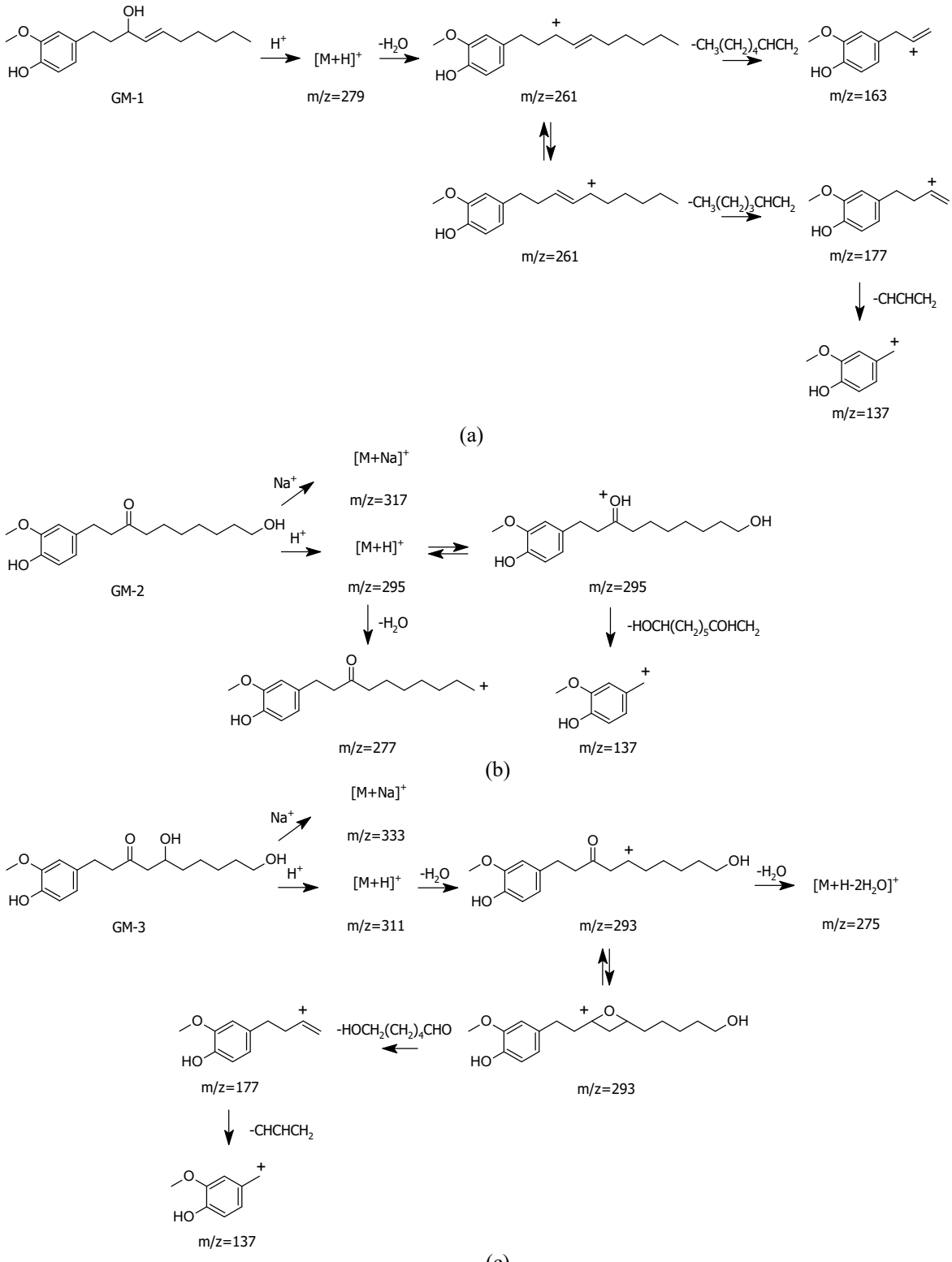


Fig. 8. (+)ESI-MS fragmentation patterns of GM-1 (a), GM-2 (b) and GM-3 (c).

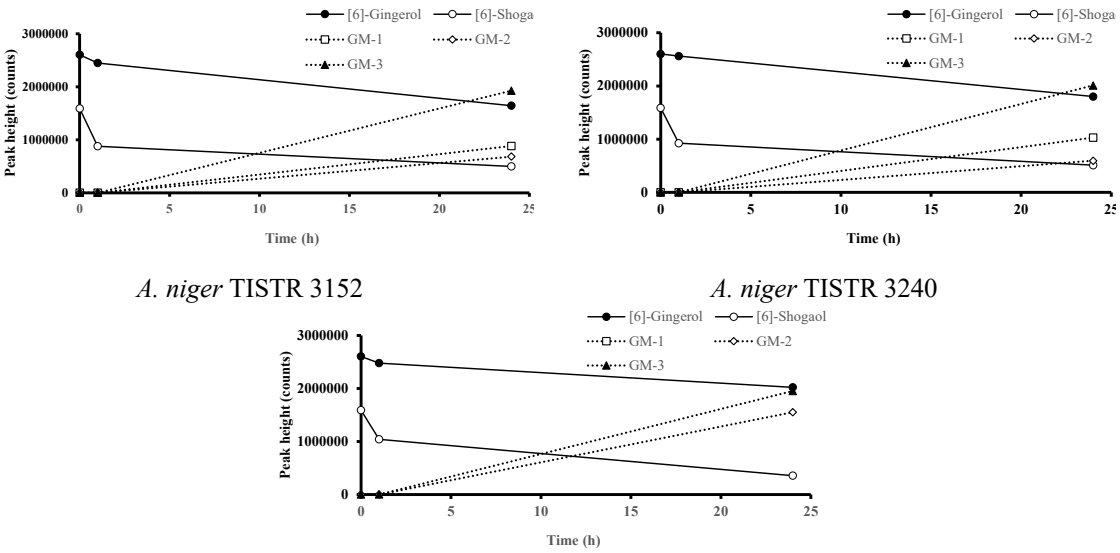


Fig. 9. The alteration of the peak heights for the substrates ([6]-gingerol (*m*/*z* 317) and [6]-shogaol (*m*/*z* 299)) and their metabolites (GM-1 (*m*/*z* 279), GM-2 (*m*/*z* 317) and GM-3 (*m*/*z* 333)) in biotransformation samples with *A. niger* TISTR 3152, *A. niger* TISTR 3240 and *P. funiculosum* TISTR 3563 analyzed by HPLC-QTOF-MS ($n = 1$).

Table 1. Retention times and MS characterization of the gingerol-related compounds detected in the control experiment without any fungus at 1-h incubation.

Retention time* (min)	Possible compounds	Major product ion, *m*/*z* [Fragmentation] (%Relative abundance)
5.755	[6]-Gingerdiol	319.1932 $[M+Na]^+$ (100); 279.1991 $[M+H-H_2O]^+$ (12); 261.1887 $[M+H-2H_2O]^+$ (40)
6.420	[6]-Gingerol	317.1770 $[M+Na]^+$ (100); 277.1833 $[M+H-H_2O]^+$ (59)
8.197	3- or 5-Acetoxy-[6]-gingerdiol	361.2015 $[M+Na]^+$ (100); 321.2078 $[M+H-H_2O]^+$ (7); 261.1864 $[M+H-H_2O\text{-}AcOH]^+$ (11)
8.663	Diacetoxy-[4]-gingerdiol	375.1811 $[M+Na]^+$ (100); 293.1762 $[M+H\text{-}AcOH]^+$ (7)
9.630	[8]-Gingerol	345.2070 $[M+Na]^+$ (100); 305.2140 $[M+H-H_2O]^+$ (52)
10.311	[6]-Shogaol	299.1641 $[M+Na]^+$ (61); 277.1828 $[M+H]^+$ (100)
11.454	Diacetoxy-[6]-gingerdiol	403.2137 $[M+Na]^+$ (100); 321.2081 $[M+H\text{-}AcOH]^+$ (22)
11.636	Methyl-[8]-gingerol	359.2212 $[M+Na]^+$ (100); 319.2276 $[M+H-H_2O]^+$ (7)
12.621	[10]-Gingerol	373.2375 $[M+Na]^+$ (100); 333.2448 $[M+H-H_2O]^+$ (83)
13.073	Methyl diacetoxy-[6]-gingerdiol	417.2297 $[M+Na]^+$ (100); 335.2248 $[M+H\text{-}AcOH]^+$ (14)
13.347	[8]-Shogaol	327.1958 $[M+Na]^+$ (31); 305.2140 $[M+H]^+$ (100)
16.101	[10]-Shogaol	355.2268 $[M+Na]^+$ (20); 333.2354 $[M+H]^+$ (100)

*The retention times of the compounds determined from the EIC at specified *m*/*z* with the abundance 100%

After 24-h fermentation with the five fungal species, it is interesting to note that the levels of all gingerol-related compounds used as substrates were likely to decrease corresponding to the control experiment (Table 2), except methyl-[8]-gingerol from *M. purpureus* TISTR 3385. Among the homologous series of both gingerols and shogaols in the ginger extract, there were no GMs that originated from [8]- and [10]-analogs, according to the specific chemical reactions for the biotransformation of [6]-gingerol and [6]-shogaol to GM-1, GM-2 and GM-3. After prolonged fermentation, the conversion of these substrates to intermediate metabolite molecules such as γ-lactone, 6-(3′-methoxy-4′-hydroxyphenyl)-4-hydroxy-hexanoic acid and homovallic acid, and subsequent further metabolization to the final products, carbon dioxide and water were observed [11, 12]. In the case of gingerdiols including diacetoxy-[6]-gingerdiol, [6]-gingerdiol, etc., that were also found in the fermentation, there have been no reports on the biotransformation of these compounds by fungal species. Therefore, it is reasonable to conclude that the metabolites, except GM-1, GM-2 and GM-3 did not accumulate enough to be detected due to their rapid metabolism and use as alternative carbon sources for cell growth.

Table 2. The relative contents of the major compounds for the biotransformation of ginger extract with different fungal strains at 24-h fermentation.

Major compounds	Relative contents (%)					
	Control	*A. niger* TISTR 3152	*A. niger* TISTR 3240	*A. flavas* TISTR 3041	*M. purpureus* TISTR 3385	*P. funiculosum* TISTR 3563
[6]-Gingerdiol	100.0	67.7	74.1	86.9	84.7	80.5
[6]-Gingerol	100.0	63.2	69.2	82.2	92.3	77.6
3- or 5-Acetoxy-[6]-gingerdiol	100.0	60.7	60.0	49.9	57.4	41.0
Diacetoxy-[4]-gingerdiol	100.0	61.0	60.2	57.7	41.9	52.4
[8]-Gingerol	100.0	6.6	8.5	21.3	57.3	10.8
[6]-Shogaol	100.0	31.4	32.1	15.2	17.6	22.3
Diacetoxy-[6]-gingerdiol	100.0	38.3	37.0	50.4	85.0	33.4
Methyl-[8]-gingerol	100.0	50.3	48.6	65.4	139.9	43.9
[10]-Gingerol	100.0	3.0	2.8	8.4	69.1	3.9
Methyl diacetoxy-[6]-gingerdiol	100.0	11.3	10.3	12.7	65.9	8.8
[8]-Shogaol	100.0	2.2	1.7	2.3	6.8	1.7
[10]-Shogaol	100.0	2.0	1.8	2.8	30.1	1.9

* The percentage of relative contents for the gingerol-related compounds was calculated by comparing the individual peak heights from the fermented samples with those of the control experiment.

4. Conclusion

The ginger extract containing phenolic compounds with related chemical structures including [6]-gingerol and [6]-shogaol, were simultaneously transformed by fungus. The isolation and purification steps for starting materials were skipped before the biotransformation. The metabolites were characterized by HPLC-QTOF-MS according to previous data regarding fragmentation patterns. Three known metabolites were produced by *A. niger* and *P. funiculosum*, and no metabolites were observed from the homologous series of gingerols and shogaols,

except [6]-gingerol and [6]-shogaol. Some metabolic products may not have been detected since the initial substrate for biotransformation had a very low concentration. This suggests that the GM profiles from the biotransformation study generally depended on the periods of fermentation and the species of fungus used. The pharmacological potential of GMs derived from biotransformation by fungi is a promising area for future research.

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