Fungal-Stressed Germination of Black Soybeans Leads to Generation of Oxooctadecadienoic Acids in Addition to Glyceollins

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Microbial-stressed germination of black soybeans leads to generation of a group of oxylipins, oxooctadecadienoic acids (KODEs, including 13-Z,E-KODE, 13-E,E-KODE, 9-E,Z-KODE, and 9-E,E-KODE), and their respective glyceryl esters in addition to glyceollins, a known phytoalexins present in wild and fungi-infected soybeans. Four fungi, *Aspergillus niger*, *Aspergillus oryzae, Rhizopus oligosporus*, and white rice yeast (*Aspergillus niger wry*), were applied to compare their efficiency on inducing these compounds during black soybean germination. Overall, *R. oligosporus*, the starter culture used in tempeh fermentation, gives the highest amounts of KODEs and glyceollins. The glyceollins and KODEs were isolated by preparative HPLC, and the structures were determined by ^{1}H NMR, UV–Vis, and MS spectra. On the basis of the unequal distribution of the KODEs isomers, an enzymatic reaction, instead of a nonenzymatic free radical chain reaction, is responsible for their formations. Together with other oxylipins and glyceollins, the KODEs may contribute to the soybean's defensive response to fungal infection via reaction with protein thiol groups and cell membranes. The stress-germinated black soybeans may be used as ingredients for further processing of novel functional food products with unique nutritional and flavor profiles.

KEYWORDS: Black soybeans; oxooctadecadienoic acids; glyceollins; phytoalexins

INTRODUCTION

Black soybean (*Glycine max* (L.) Merrill) is a soybean cultivar with a black seed coat that belongs to the family Leguminosae. It has been used in traditional Chinese medicine and as functional food since ancient time, yet it is not as widely produced and consumed as typical yellow soybeans. A few studies demonstrated that black soybeans may have more bioactive micronutrients than the yellow counterpart (*1*). A high molecular weight polysaccharide of black soybean with an α-linked glucan structure was found to provide immune regulation and antitumour effects (*2*). Black soybeans and germinated seeds possess greater antioxidant capacity than yellow soybeans (*3*). With its higher polyphenol content, black soybeans were shown to be more effective in inhibiting low density lipoprotein (LDL) oxidation and may be more effective in preventing oxidative stress (*4*). Black soybean containing herbal prescriptions can increase the number of circulating white blood cells in leukopenic patients (*5*). Despite the potentially greater health benefits of black soybeans, they are not fully explored as ingredients for functional soy products.

Phytoalexins, considered as plant antibiotics (*6*), are small molecule compounds synthesized by plants to defend against microbial infection. There are two types of phytoalexins, constitutive and inducible (*7*). Phytoalexins are typically toxic to microbes but might not be so for humans. In fact, phytoalexins and their benefits on human health are just immerging as a rewarding research area. This is best demonstrated by resveratrol, a phytoalexin found in grapes and herbal plants. Resveratrol has antioxidant, anti-inflammation, and anticancer activity (*8*). In addition, it acts as a calorie restriction (CR) mimetic that extends the life span of laboratory animals (*9, 10*). Calorie restriction refers to reduction of calorie intake by 30–50% of the normal intake level without causing malnutrition. It is suggested that plant synthesizes phytoalexins to activate sirtuin pathways (*11*) and may also activate animal sirtuins and consequently exert the benefits of CR (*12*). Glyceollins are a group of inducible phytoalexins generated by soybeans under fungal, chemical, and environmental stress (*13*), and they have been shown to inhibit fungal (*Phytophthora megasperma* var. sojae) growth (*14*). Glyceollins have higher activity as antiestrogens than daidzein, the glyceollins precursor, in blocking the growth and spread of gynecologic cancers (*15*). Although glyceollins may render soybeans added health benefits, they are not present in significant amounts in soy products in the market. This is because the cultivated soybeans typically do not encounter pathogens that elicit the production of glyceollins. In the pursuit to develop novel and more nutritious fermented food derived from black soybeans and also to eliminate the undesired antinutrients, we inoculated the germinating black soybeans with

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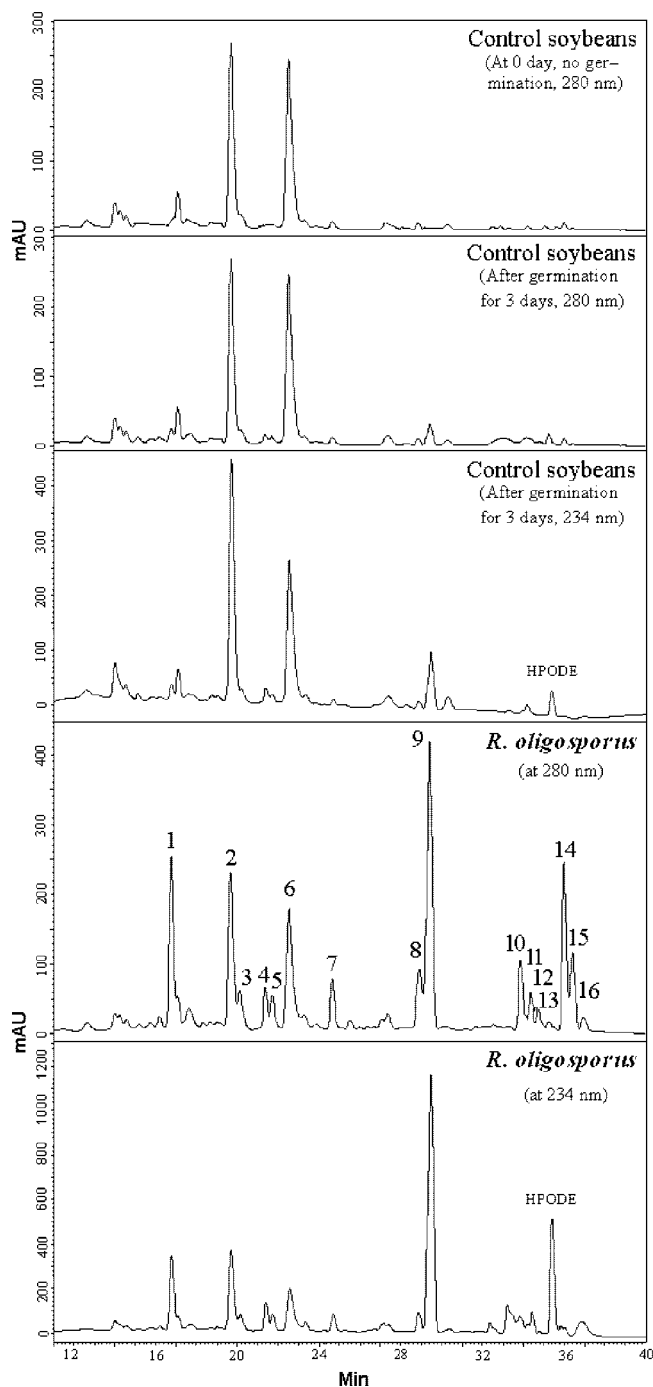
Figure 1. HPLC chromatograms of ungerminated black soybeans, germinated black soybeans after 3 days without fungal inoculation, and 3 day germinated black soybeans with fungal inoculation. Peak identities: 1, genistin; 2, malonyl daidzin; 3, malonyl glycitin; 4, daidzein; 5, glycitein; 6, malonyl genistin; 7, genistein; 8–9 glyceollins; 10–13, KODE glyceryl esters; 14–16, KODEs.

microbes and studied the changes of secondary metabolites upon such treatment. We discovered, among other things, the black soybean produced not only large amounts of glyceollins but also oxooctadecadienoic acids (KODEs) and their glyceryl esters when the beans were placed under the fungal stress. Documented herein are our findings.

EXPERIMENTAL SECTION

Materials and Instruments. The tempeh starter culture, *Rhizopus oligosporous*, was bought from PT. Aneka Fermentasi Industri (Bandung, Indonesia). The identity of the fungus is confirmed at the School of Technobiology at Indonesia Catholic University (Jakarta, Indonesia). *Aspergillus oryzae* and *Aspergillus niger* were cultivated in house. White rice yeast and black soybeans were a product of China and purchased from a local supermarket. The identity of the species was confirmed to be *Aspergillus niger wry*. HPLC analysis was carried out on a Waters HPLC system with a 2996 PDA detector. ^{1}H NMR spectra were recorded in deuterated chloroform with a Bruker AC300 spectrometer. LC/MS spectra were acquired using a Finnigan/MAT LCQ ion trap mass spectrometer (San Jose, CA) equipped with a TSP 4000 HPLC system, which includes a UV6000LP PDA detector, P4000 quaternary pump, and AS3000 autosampler. The heated capillary and spray voltage were maintained at 250 °C and 4.5 kV, respectively. Nitrogen is operated at 80 psi for sheath gas flow rate and 20 psi for auxiliary gas flow rate. The full scan mass spectra from m/z 50 to 2000 were acquired both in positive and in negative ion modes with a scan speed of one scan per second.

Black Soybean Germination and Fungal Inoculations. *R. oligosporus* culture powder (1.0 g) was suspended in sterile deionized water (15.0 mL) to obtain a spore suspension of 1×10^8 mL^{-1}. Other fungi were grown at 25 °C in the dark on a tube with potato dextrose agar. Conidia of each fungi were suspended in sterile water to obtain a spore suspension of 1×10^8 conidia mL^{-1}. The fungal inoculations were carried out on the black soybean cotyledons according to the method reported by Boué (16) with some modifications. Black soybean seeds (200 g) were surface-sterilized in 400 mL of 70% ethanol for 3 min and then rinsed three times with water (500 mL) to wash away the ethanol. Seeds were soaked in sterile water for 10 h. The water was drained from the soaked beans, and each bean was peeled into two pieces without spoiling the radicals. The prepared fungal culture suspension was inoculated onto the beans and mixed well (15 mL of each fungal suspension inoculated 200 g of black soybeans). The inoculated beans were placed on a sterile container (30 × 50 cm) lined with two autoclaved filter papers moistened with 30 mL of sterile water. The containers were sealed with Parafilm and incubated for 3 days at 25 °C in the dark. Some black spots formed on the germinated bean seeds with short sprouts. Control black soybeans were also prepared with identical procedures except that no fungus was inoculated onto the beans.

Compound Identification and Isolation. Soybean samples were homogenized in 80% ethanol (7 mL/g of seeds) and then heated at 50 °C for 1 h in a water bath with continuous shaking. After the samples cooled, the mixtures were centrifuged at 14000g for 15 min. The supernatant was collected and filtered through a PTFE membrane (0.45 μM) before injecting into an HPLC instrument with detector wavelengths set at 234 and 280 nm. The separation was accomplished on a Shimadzu ODS-VP (4.6 × 250 mm, 5 μm i.d.) column with water (A) and acetonitrile (B) as the mobile phase. The gradient was as follows: 0–1 min, A 100%; 1–17 min, A from 100% to 55%; 17–27 min, A from 55% to 10%; 27–33 min, A 10%; 33–35 min, A from 10% to 100%; 35–40 min, A 100%. For preparative scale operations (0.5 kg of soybean seeds per batch), the ethanol-extracted supernatant was concentrated to a small volume (~10 mL, contains mainly water). An equal volume of petroleum ether (bp 35–60 °C) was added to the concentrated supernatant and extracted twice for 1 h with vigorous shaking. The ether layers of the two extractions were combined and evaporated by a rotary evaporator. The concentrated residue was transferred to a silica gel column (20 × 4.5 cm, silica gel 60) for crude separation.
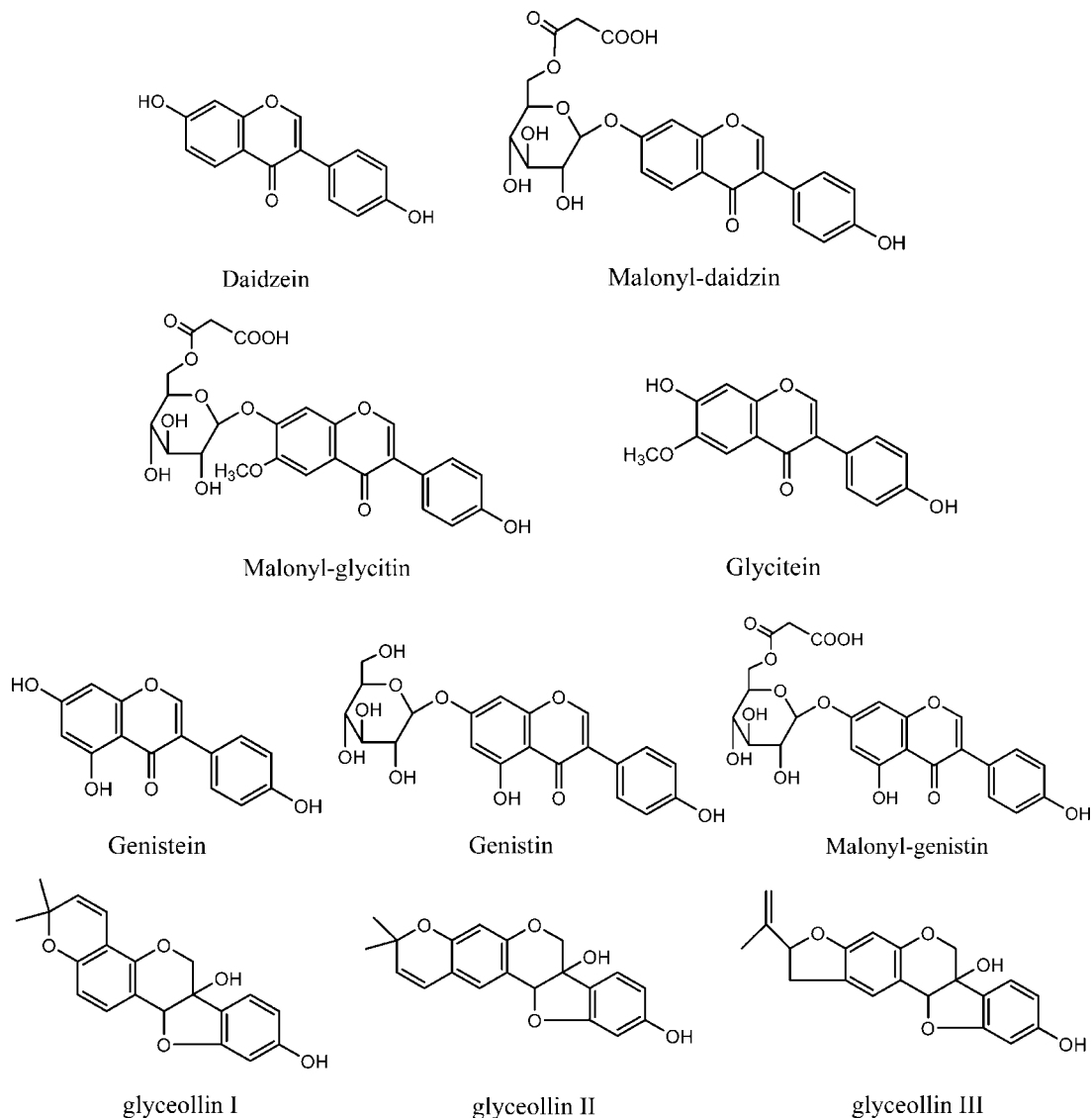
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Figure 2. The chemical structures of the isoflavones and glyceollins identified in the HPLC chromatogram.
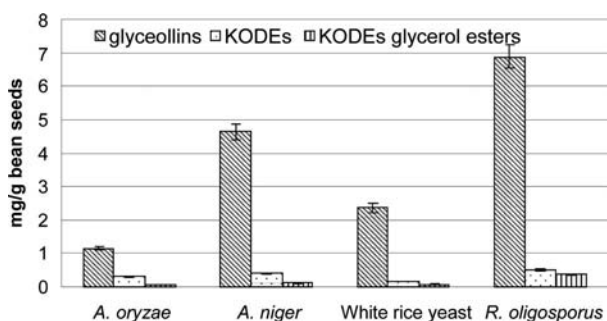

Figure 3. Comparison of the concentrations of glyceollins, KODEs, and KODE glyceryl esters in the black soybeans stressed by four different fungal strains with a 3 day germination. All runs were in triplicate, and the results are expressed as mean ± relative standard deviation.

The column was pre-equilibrated with hexane and then successively eluted with hexane (300 mL) and a hexane/ethyl acetate mixture (300 mL each of 7:3, 6:4, 5:5, 4:6, 2:8, and 0:10) at a flow rate of 4 mL/min. Each fraction was collected (20 mL) and concentrated through rotary evaporation. After HPLC identification, the fractions containing the interested compounds are combined and further purified by semipreparative HPLC using a YMC-Pack ODS-AM C18 (10 × 250 mm; 5 μm) column. Elution was carried out at a flow rate of 5.0 mL/min

with the following solvent system and gradient: water (A); acetonitrile (B); 0% B to 45% B in 17 min, then 45% B to 90% B in 10 min, followed by holding at 90% B for 6 min. The collected pure compounds (∼10 mg) were identified via MS and ^{1}H NMR spectroscopy.

RESULTS

Fungal Elicitation of Glyceollins and Oxooctadecadienoic Acids (KODEs). In an attempt to elicit glyceollins and improve the nutritional profiles, germinating black soybeans were inoculated with *A. niger*, *A. oryzae*, *R. oligosporus*, and white rice yeast (*A. niger wry*). The ethanol extracts of the resulting beans are analyzed by HPLC and chromatograms of control and *R. oligosporus* stressed samples (at 234 nm and 280 nm, respectively) are shown in **Figure 1**. The identities of the peaks were verified by LC/MS in combination with standard isoflavones and KODEs (vide infra) (structures shown in **Figure 2** and **5**). It is remarkable that the unstressed germination of black soybeans did not lead to major changes of the HPLC profile compared to the ungerminated beans. In both germinated and ungerminated control samples, the malonyl daidzin (peak 2) and malonyl genistin (peak 6) are the major compounds along with comparable minor peaks (**Figure 1**, top two traces). Only an insignificant increase of the glyceollins (peak 9 and the
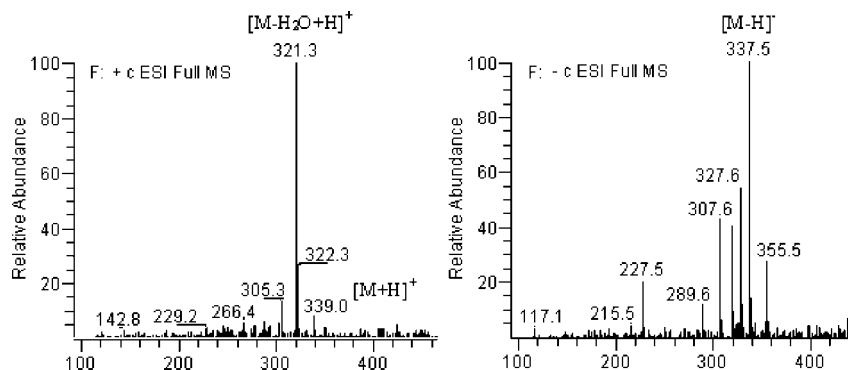
Figure 4. A representative ESI (positive and negative ion) mass spectrum of glyceollin I.

associated peaks nearby) and KODEs (peak 14) is seen in the control samples, which may due to minor spoilage occurring during germination. Under fungi-stressed germination, drastic changes of the glyceollins and KODEs as well as KODEs glyceryl esters are detected and the profiles are also highly dependent on the types of fungi. **Figure 3** shows a comparison of the concentrations of glyceollins, KODEs, and KODE glyceryl esters (peaks 10–13 in **Figure 1**) in the four fungi-stressed germinating samples. With *A. niger* or *A. oryzae* as the elicitors, very small amounts of KODE glyceryl esters were detected, with *A. oryzae* eliciting the lowest amount of glyceollins. This result is quite different from that of the yellow soybeans reported. *A. oryzae* and *A. niger* elicited comparable and significant amounts (660 vs 623 μg/g of fresh weight) of glyceollins from soybean cotyledons of Pioneer 95B41 (*13*). White rice yeast, which is a common starter culture readily available in Singapore for rice wine fermentation, also gave only moderate amounts of glyceollins and KODEs. The different bean species, the strain of fungi used, and the variations in the amount of elicitor applied may also contribute to the differences observed. *R. oligosporous*, which is normally used to ferment cooked soybeans to make tempeh in Indonesia, is a far more effective elicitor than *A. niger* and *A. oryzae*. The amount of the glyceollins elicited from black soybeans by *R. oligosporous* was close to 7 mg/g (dry matter) in addition to the highest amounts of KODEs and KODE glyceryl esters. Remarkably, the precursors to glyceollins (*16*), malonyl daidzin and daidzein, do not decrease while the glyceollins are accumulated. This apparent increase of glyceollins may be accounted for if the soybean seeds synthesize glyceollins from other precursors such as phenylalanine. In order to confirm whether glyceollins, KODEs, and their glyceryl esters were produced by fungi themselves, the mycelia and conidia of each strain grown in the soybeans were collected and treated with the same extraction method, but none of these compounds was detected by HPLC chromatography. To rule out the possibility that the KODEs were produced by the fungal action on soybeans, the thermal-deactivated soybeans were inoculated with *R. oligosporous* for 3 days and the resulting soybeans were analyzed to find no significant amount of KODEs. Therefore, glyceollins, KODEs, and their glyceryl esters were produced by the soybeans as a response to the fungal stress.

Characterization of Glyceollins, KODEs, and KODE Glyceryl Esters. The identities of the glyceollins are derived from MS^n spectroscopy (**Figure 4**). The ESI-MS of peak 9 exhibit m/z 339 (cation) and 337 (anion) and the fragmentation pattern of secondary mass features loss of a water molecule (m/z 321). These data are in agreement with literature (*14*) for glyceollin I. So far, there are five glyceollins reported from elicitation of soybeans using cupric chloride (*13*). In fungal-

Table 1. Negative Ions (*m/z*) and Proposed Structures for Fragments of KODEs

	m/z (relative abundance)	
fragments	**13-**Z, E-KODE, 13-E,E-KODE	**9-**E, Z-KODE, 9-E,E-KODE
[M − H]⁻	293 (20)[a]	293 (60)
[M − OH − H]⁻	275 (48)	275 (28)
		257 (18)
[M − CO₂ − H]⁻	249 (58)	249 (52)
[M − CH₃(CH₂)₄CHCH₂ − H]⁻		197 (18)
[M − CH₃(CH₂)₂CHCH₂ − CO − H]⁻	195 (4)	
		185 (60)
[M − CH₃(CH₂)₂CHCH₂ − CO₂ − H]⁻	179 (22)	
		177 (16)
[M − CH₃(CH₂)₂CHCH₂COCH₂CH₂ − H]⁻	167 (18)	167 (8)
		149 (22)
[M − CH₃(CH₂)₄(CHCH)₂CHO − H]⁻		141 (8)
[M − CH₃(CH₂)₄CHCHCHCH₂CO₂H]⁻		125 (10)
[CH₃(CH₂)₄CHCHCHCH₂ − H]⁻	113 (58)	113 (60)

[a] Abundances for secondary and tertiary ESI-MS fragment ions at voltage 35.00 V.

stressed soybeans, only glyceollins I, II, and III are detected, as is the case in our study. Glyceollin I is consistently dominant among the three isomers.
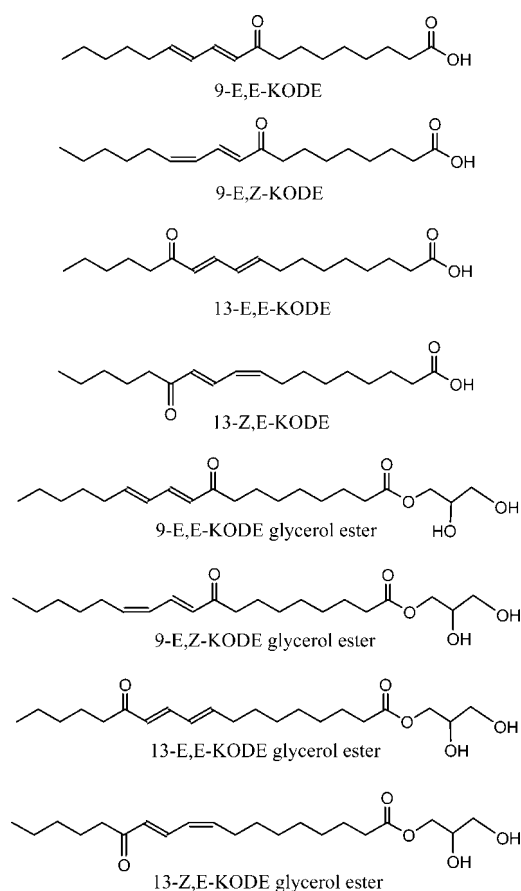
Under negative ion mode, peaks 14–16 have the same molecular ion of m/z 293, indicating that they were four isomers. From an accurate molecular weight of 294.2196, the molecular formula was calculated to be $C_{18}H_{30}O_3$. Secondary and tertiary MS were further conducted to get full fragmentation patterns (**Table 1**), which were in agreement with the patterns of KODEs reported (*18*). Moreover, ^1H NMR results of the four isomers were consistent with those of KODEs published previously by Kawagishi and co-workers who isolated the KODEs from mushrooms (*19*). Accordingly, the structural assignments of these compounds were identified as 13-Z,E-oxooctadeca-9,11-dienoic acid (13-Z,E-KODE), 13-E,E-oxooctadeca-9,11-dienoic acid (13-E,E-KODE), 9-E,Z-oxooctadeca-10,12-dienoic acid (9-E,Z-KODE), and 9-E,E-oxooctadeca-10,12-dienoic acid (9-E,E-KODE), respectively. In general, mass spectra of the KODEs revealed a common molecular ion at m/z 293 for [M − H]⁻. Dehydration from the molecular ion led to an m/z 275 fragment. The ion at m/z 249 was a decarboxylation fragment. For 13-Z,E-KODE and 13-E,E-KODE, characteristic fragments at m/z 195 and 179 were obtained from scission between C13 and C14. Successive fragmentations of 195 led to characteristic ions at m/z 167 and 113 due to the loss of ethylene or CO. Typical ions for 9-E,Z-KODE and 9-E,E-KODE were detected at m/z 197, 185, 177,149, 141, 125, 123, and 113. Cleavage between C11 and C12 may result in m/z 197 while cleavage between C9 and C10 may produce m/z 123. In agreement with the MS

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Table 2. ^{1}H NMR Spectral Data for KODEs^{a,b}

position	13-*Z,E*-KODE	13-*E,E*-KODE	9-*E,Z*-KODE	9-*E,E*-KODE
2	2.32 (2H,t,J = 6.7 Hz)	2.32 (2H,t,J = 6.7 Hz)	2.32 (2H,t,J = 6.7 Hz)	2.32 (2H,t,J = 6.7 Hz)
3	1.5–1.7 (4H, m)	1.5–1.7 (4H, m)	1.5–1.7 (4H, m)	1.5–1.7 (2H, m)
4–6, 16, 17	1.20–1.40 (12H, m)	1.20–1.40 (12H, m)	1.20–1.40 (10H, m)	1.20–1.40 (2H, m)
7	1.20–1.40 (12H, m)	1.20–1.40 (12H, m)	1.6 (4H, m)	1.6 (2H,m)
8	2.3 (2H, dt)	2.16 (2H, m)	2.51 (2H, t, J = 7.3 Hz)	2.51 (2H, t, J = 7.3 Hz)
9	5.91 (1H, dt)	6.15 (2H, m)		
10	6.12 (1H, dd)	6.15 (2H, m)	6.16 (1H, m)	6.05 (1H, d, J = 15.3 Hz)
11	7.50 (1H, m)	7.11 (1H, m)	7.50 (1H, m)	7.11 (1H, m)
12	6.16 (1H, m)	6.05 (1H, d, J = 15.3 Hz)	6.12 (1H, dd)	6.15 (2H, m)
13			5.91 (1H,dt)	6.15 (2H, m)
14	2.51 (2H, t, J = 7.3 Hz)	2.51 (2H, t, J = 7.3 Hz)	2.3 (2H,dt)	2.16 (2H, m)
15	1.6 (2H, m)	1.6 (2H, m)	1.42 (2H, m)	1.42 (2H, m)
18	0.87 (3H, t, J = 6.9 Hz)	0.87 (3H, t, J = 6.9 Hz)	0.87 (3H, t, J = 6.9 Hz)	0.87 (3H, t, J = 6.9 Hz)

^{a} Chemical shift, δ, ppm. ^{b} Analytes were dissolved in CDCl$_3$, chemical shifts were referenced to CHCl$_3$ (7.26 ppm).

Figure 5. The structures of oxooctadecadienoic acids (KODEs) and their respective glyceryl esters.

Figure 6. The proposed pathways for enzymatic formation of KODEs (illustrated using 13-*E,E*-KODEs as an example) via linoleic acid in fungal-stressed germination of black soybeans.

data, the ^{1}H NMR data of isolated KODEs are shown in **Table 2**. The identity and quantification of the KODEs were further confirmed by HPLC chromatograms spiked with authentic samples obtained commercially.

Peaks 10–13 to gave the same molecular ions of *m/z* 367, suggesting that they were four isomers. The molecular formulas were determined by high-resolution MS as C$_{21}$H$_{36}$O$_5$ (for *m/z* of 368.2564). A daughter ion of *m/z* 293 was detected for all four compounds. Secondary and tertiary MS were further conducted to get full fragmentations identical to those for KODEs shown in **Table 1**. Therefore, compounds for peaks 10–13 are derivatives of KODEs, with mass difference of 74 being a glyceryl moiety, CH$_{2(3')}$OH–CH$_{(2')}$OH–CH$_{2(1')}$–. This moiety assignment is confirmed from the NMR data of isolated samples, which gave signals at 4.12 (H$_{1'a}$), 4.17 (dd, J = 11.6, 6.3 Hz, H$_{1'b}$), 3.90 (m, H$_{2'}$), 3.57 (dd, J = 11.1, 5.5 Hz, H$_{3'a}$),

3.67 (dd, J = 11.1, 3.4 Hz, H$_{3'b}$). Therefore the KODEs are not attached to the C2′ hydroxyl groups which would give rise to compound with mirror plane and the ^{1}H NMR spectra of C$_{1'}$ and C$_{3'}$ would be identical (**Table 2** and **Figure 5**).

DISCUSSION

It is extensively documented that glyceollins were formed when soybeans are stressed either with microbe or with merely an inorganic salt such as cupric chloride (*16*). We demonstrated that inoculation of the germinating black soybeans also elicit the formation of glyceollins. The amount of glyceollins formed is highly dependent on the type of microbes used. Among the few elicitors we tested, *R. oligosporous* is the most effective elicitor with over 7 mg/g (dry soybean) glyceollins formed. In addition, the isoflavone profiles are drastically different from the controlled sample (**Figure 1**), which showed only minor changes before and after germinations for 3 days. Formation of KODEs under stress germination of soybeans was unprecedented, and they may be considered as phytoalexins according to the broader definition of phytoalexins as small molecular compounds generated by plants under stress (*20, 21*). In fact, most oxylipins can impair the growth of plant microbial pathogens. 9-KODEs, at concentration of 100 μM, are highly effective in inhibiting *Phytophthora parasitica nicotianae* and *Cladosporium herbarum* (*22*). The concentrations of the KODEs

in our case were much lower taking into account that the whole soybean matters; however, the localized KODEs concentration in the soybean tissues with direct contact with the fungi may be high enough for effective inhibition of the growth of the fungi. The formation of KODEs could be resulting from oxidative burst, an early plant response to pathogen infection with rapid and transient production of a large amount of reactive oxygen species (ROS), primarily superoxide (O_2^-) and hydrogen peroxide (H_2O_2). Guo and co-workers found that the treatment of soybean cell suspension cultures with avirulent pathogens would trigger an oxidative burst resulting in expressions of the isoflavonoid phytoalexin glyceollins (*23–25*). One might suspect that the excessive radical activity might trigger lipid peroxidation and lead to formation of KODEs as one of the secondary products. However, the unequal distribution of the four isomers KODEs speaks against this possibility. Instead, the linoleic acid in the soybean may be oxidized selectively by lipoxygenases to give hydroperoxyoctadecadienoic acid (HPODE). KODEs can be reduced by hydroperoxidase to hydroxyloctadecadienoic acid (HODE), the latter dehydrogenated to give KODEs (**Figure 6**). We have independently oxidized methyl linoleate with 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN, a lipid soluble radical initiator) and found that the resulting hydroperoxides (HPODE) have equal distribution of four products corresponding to four isomers of HPODEs consistent with a literature report (*26*). In the germinated soybean samples, only one major HPODE peak was detected with retention time at 35.5 min (234 nm, **Figure 1**). The region- and stereospecificity of lipoxygenases are highly dependent on the plant origin. In soybean, it predominantly produces 13-*Z,E*-HPODE, whereas in tomato and corn only 9-*E,Z*-KODE was formed (*27–29*). Although lipoxygenase was originally contained in the control black soybeans (*30*), HPODEs were not detected in significant amount. The increased formation of HPODE may be needed in infected soybeans for defense of microbial invasions, possibly the HPODE acts as free radical precursor when it is one-electron reduced to form alkoxy radicals (RO$^•$) or hydroxyl radicals (HO$^•$) either by hydroperoxidase or by transition metal ions such as ferrous and cuprous ions. These radicals are highly reactive and can inactivate fungi. In this regard, there is a remote similarity to human immune response to bacteria infections, in which the macrophages generate free radicals to destroy the invading bacteria.

Physiologically, 13-KODEs may also play certain roles in cell differentiation (*31*). With extended unsaturated ketonic conjugation, 13-KODEs are potent electrophiles that are known to react with biologically common nucleophiles like glutathione and the thiol residue of protein to form bioconjugates (*32*). 13-KODEs and their glyceryl and ethyl esters show inhibition activity of aldehyde dehydrogenase *in vitro* (*19*) and inhibitor for acetyl CoA carboxylase, which is one of the key enzymes in fatty acid biosynthesis. As a result, KODEs could be able to retard fat accumulation (*34*). Further research is needed to understand (a) if the oxylipins including KODEs are detrimental to human health when they are consumed and (b) how the KODEs may alter the flavor profile of the finished food product.

In conclusion, we found that the black soybeans respond to fungal stress by generating much higher amounts of glyceollins and KODEs, which are needed for defense of the bean seeds against pathogens. Further research is warranted to understand the exact roles of KODEs and their precursors in soybean defense. The infection of microbes greatly alters the metabolite profiles in soybeans. To comprehensively characterize the metabolites, metabonomic analysis through comparison of the stressed and unstressed soybean germination products will give complete pictures and likely uncover more lipid oxidation products and other phytoalexins beyond glyceollins (*34*). Using food-grade microbes to elicit the biosynthesis of glyceollins alters the secondary metabolite profiles of the soybean and hence the nutritional values of the subsequent food products made from the bean seeds.

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