Furan, phenolic, and heptelidic acid derivatives produced by *Aspergillus oryzae*

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Abstract Eleven compounds, including a new sesquiterpene, were isolated from the culture medium of *Aspergillus oryzae* incubated with capsaicin. The structure of the new compound was determined to be 1,3,5a,6,7,8,9,9a-octahydro-9-hydroxy-9-(hydroxymethyl)-6-isopropyl-1-oxobenzo[c]oxepine-4-carboxylic acid, a heptelidic acid derivative. In addition, 10 known compounds were identified, namely 5-(hydroxymethyl)-3-furancarboxylic acid (flufuran), 3-hydroxypropanoic acid, 5-(hydroxymethyl)-2-furancarboxylic acid, 2-(4-hydroxyphenyl)-ethanol, 4-hydroxybenzoic acid, vanillic acid, 3,4-dihydroxybenzoic acid, 2-furanol, hydroheptelidic acid, and trichoderonic acid A, using spectroscopic data from nuclear magnetic resonance and electrospray ionization–mass spectroscopy.

Keywords: *Aspergillus oryzae*, metabolites, sesquiterpene, heptelidic acid, phenolics

Introduction

Gochujang, a fermented red-pepper paste, is widely consumed as a sauce in Korea and used as a spicy seasoning in Korean cooking (1). Gochujang is prepared by fermenting a mixture that primarily comprises red hot pepper powder, waxy rice flour, fermented soybean, and koji (1). Gochujang has a unique sweet, savory, and pungent flavor. Gochujang has been reported to have various biological effects, such as antiobesity (2,3), antitumor (4), insulin sensitizing (5), and anticancer (6) activities. The flavor and biological effects of gochujang are dependent on its primary ingredients, microorganisms, and the extent of fermentation (7,8). Red pepper and fermented soybeans are the major ingredients used in the preparation of gochujang; it also contains various flavorings and beneficial compounds such as capsaicin derivatives and flavonoids (9,10). Compounds found in the primary ingredients are metabolized by microorganisms during the fermentation process (1,7,8), and the metabolites contribute to the quality, taste, and biological effects of gochujang.

Recently, in a previous study, we identified various biological compounds, including flavonoid glycosides and diterpene glycosides, in gochujang fermented by *Aspergillus oryzae*, which is often used to make mass-produced gochujang (11,12). However, various flavonoid glycosides in the primary ingredients were not metabolized during

the fermentation of gochujang (13). We also demonstrated that *A. oryzae* produces β -glucosidase, but its activity is strongly inhibited by the ingredients in gochujang (13). Interestingly, fermented gochujang has a less pungent taste and is lower in capsaicin than red hot pepper. This may be related to the capsaicin metabolism during gochujang fermentation. However, gochujang capsaicin metabolites have not yet been isolated. Therefore, in our previous study (14), to identify capsaicin metabolites produced during gochujang fermentation, capsaicin was cultured with *A. oryzae* in potato dextrose medium. Five capsaicin metabolites, including two new capsaicin metabolites, were identified from the capsaicin culture. Using high-performance liquid chromatography–electrospray ionization mass spectrometry (HPLC–ESI-MS) analysis, we confirmed that four capsaicin metabolites were present in gochujang. In the course of investigations the capsaicin metabolites, 11 other compounds, including a new sesquiterpene lactone (a heptelidic acid derivative), were isolated. In this study, we describe the isolation and structural elucidation of 11 compounds isolated from the capsaicin-containing culture medium metabolized by *A. oryzae*.

Materials and Methods

Chemicals and strains Capsaicin (purity, 50%) was purchased from

Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The detailed methods for purification of capsaicin via preparative HPLC and confirmation of its purity ($\geq 98\%$) via nuclear magnetic resonance (NMR) and MS analyses have been reported previously (14). Methanol (MeOH) used in the HPLC analysis was purchased from Fisher Scientific Korea Ltd. (Seoul, Korea), and deuterium methanol (CD_3OD), which was used as a solvent in the nuclear magnetic resonance (NMR) analysis, containing 0.03% tetramethylsilane (TMS) was purchased from Acros Organics (Morris Plains, NJ, USA). *A. oryzae* (KCCM 11372) was obtained from the Korean Culture Center for Microorganism (KCCM, Seoul, Korea). Potato dextrose broth (PDB) was purchased from Difco Co. (Detroit, MI, USA) and was used to culture *A. oryzae*.

Incubation of *A. oryzae* The detailed procedure for incubation of *A. oryzae* with capsaicin has been reported previously (14). Briefly, purified capsaicin (200 mg) was added to PDB (2 L), inoculated with a 5% (v/w) spore suspension of *A. oryzae*, and incubated for 14 days at 30°C and 120 rpm in a shaking incubator to obtain the cultured capsaicin broth.

Extraction and purification The cultured capsaicin broth was centrifuged for 15 min at 4°C and 890×g. The supernatant was suspended in distilled water (2 L) and extracted using 4 L of ethyl acetate (EtOAc, three times). The EtOAc fraction was evaporated under a vacuum. The concentrate was fractionated via column (3.0×80 cm) chromatography using octadecylsilane (ODS; 70–230 mesh, YMC, Kyoto, Japan) and eluted using $\text{H}_2\text{O}/\text{MeOH}$ (7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 0:10, v/v, each 300 mL). Each fraction obtained from column chromatography was subjected to silica gel thin-layer chromatography (TLC; silica gel 60F254, 0.25 mm thickness; Merck, Darmstadt, Germany), and the plate was developed using a mixture of EtOAc/acetic acid/chloroform (CHCl_3) (5:1:2, v/v/v). To group the fractions, the spots were detected using ultraviolet (UV) light (254 nm) and a 1% cerium (IV) sulfate ethanol solution spray. Fractions were purified using HPLC with a $\mu\text{Bondapak}$ (C18, 10 μm , 7.8×300 mm, Waters, Milford, MA, USA). The flow rate was 5.0 mL/min, and the compounds were monitored at 254 or 210 nm. The mobile phase was subjected to a MeOH gradient [10% MeOH→50% MeOH (40 min) for fraction B (15.3 mg), 20% MeOH→60% MeOH (40 min) for fraction C (11.5 mg), 40% MeOH→80% MeOH (40 min) for fraction F (8.4 mg), and 50% MeOH→80% MeOH (40 min) for fraction G (4.5 mg)].

NMR and MS analysis NMR spectra were obtained using a ^{unity}INOVA 500 spectrometer (Varian, Walnut Creek, CA, USA). The isolated compounds were dissolved in CD_3OD , and the chemical shift was measured using TMS as an internal standard. Direct ESI-MS analyses were conducted using an ESI-MS spectrometer (SYNAPT G2; Waters).

Compound **6**: ¹H- and ¹³C-NMR data are listed in Table 1; ESI-MS

Table 1. ¹H- (500 MHz) and ¹³C- (125 MHz) NMR data for **6** in CD_3OD

Position	δ_{H} (int., mult., J in Hz)	δ_{C}
1	-	174.4
3a	5.22 (1H, d, 14.5)	62.7
3b	5.01 (1H, d, 14.5)	
4	-	130.8
5	7.25 (1H, d, 4.0)	146.8
5a	2.61 (1H, dt, 12.0, 4.0)	41.3
6	1.58 (1H, m)	50.0
7a	1.75 (1H, dd, 13.5, 4.0)	22.3
7b	1.28 (1H, dd, 10.0, 4.5)	
8a	2.47 (1H, td, 13.5, 3.5)	36.3
8b	1.35 (1H, m)	
9	-	74.1
9a	3.73 (1H, d, 12.0)	54.2
10	-	168.6
11	2.11 (1H, m)	28.8
12	1.00 (3H, d, 7.0)	21.8
13	0.93 (3H, d, 7.0)	15.8
14a	4.81 (1H, d, 12.5)	48.3
14b	3.91 (1H, d, 12.5)	

(negative): m/z 297.1 [$\text{M}-\text{H}$][−].

Compound **10**: ¹H-NMR (500 MHz, CD_3OD) δ 4.25 (1H, d, $J=12.5$ Hz, H-3a), 4.11 (1H, d, $J=12.5$ Hz, H-3b), 6.62 (1H, d, $J=10.5$ Hz, H-4), 2.67 (1H, m, H-5), 2.11 (1H, d, $J=11.0$ Hz, H-6), 1.77 (1H, m, H-8a), 1.30 (1H, dt, $J=13.0$, 3.5 Hz, H-8b), 2.01 (1H, td, $J=14.0$, 3.5 Hz, H-9a), 1.67 (1H, dt, $J=13.5$, 5.0 Hz, H-9b), 1.34 (1H, m, H-10), 1.79 (1H, m, H-11), 0.77 (3H, d, $J=7.0$ Hz, H-12), 0.97 (3H, d, $J=6.5$ Hz, H-13), 4.46 (1H, d, $J=10.0$ Hz, H-15a), 4.06 (1H, d, $J=10.0$ Hz, H-15b); ¹³C-NMR (125 MHz, CD_3OD) δ 170.3 (C-1), 135.1 (C-2), 57.5 (C-3), 145.6 (C-4), 41.8 (C-5), 54.0 (C-6), 76.6 (C-7), 22.1 (C-8), 33.1 (C-9), 46.7 (C-10), 29.6 (C-11), 16.0 (C-12), 22.0 (C-13), 179.6 (C-14), 76.4 (C-15); ESI-MS (negative): m/z 297.1 [$\text{M}-\text{H}$][−].

Compound **11**: ¹H-NMR (500 MHz, CD_3OD) δ 5.18 (1H, d, $J=14.5$ Hz, H-3a), 5.03 (1H, d, $J=14.5$ Hz, H-3b), 7.26 (1H, d, $J=4.0$ Hz, H-5), 2.69 (1H, dt, $J=3.0$, 4.0 Hz, H-5a), 1.53 (1H, m, H-6), 1.70 (1H, m, H-7a), 1.68 (1H, m, H-7b), 2.30 (1H, m, H-8a), 1.34 (1H, m, H-8b), 3.46 (1H, d, $J=12.5$ Hz, H-9a), 2.10 (1H, m, H-11), 0.90 (3H, d, $J=7.0$ Hz, H-12), 0.99 (3H, d, $J=7.0$ Hz, H-13), 4.18 (1H, d, $J=10.5$ Hz, H-14a), 3.75 (1H, d, $J=10.5$ Hz, H-14b), 3.39 (3H, s, $-\text{OCH}_3$); ¹³C-NMR (125 MHz, CD_3OD) δ 174.8 (C-1), 62.8 (C-3), 131.0 (C-4), 147.7 (C-5), 41.1 (C-5a), 49.6 (C-6), 22.4 (C-7), 36.4 (C-8), 74.9 (C-9), 54.1 (C-9a), 168.6 (C-10), 28.9 (C-11), 15.7 (C-12), 21.8 (C-13), 74.8 (C-14), 59.8 ($-\text{OCH}_3$); ESI-MS (negative): m/z 311.1 [$\text{M}-\text{H}$][−].

Results and Discussion

Purification and isolation Capsaicin was metabolized by *A. oryzae* for 14 days in PDB medium. After centrifugation, the supernatant was partitioned using EtOAc. In a previous study conducted by our group (14), five capsaicin metabolites were isolated and identified

Fig. 1. Purification of compounds from *Aspergillus oryzae* culture medium with capsaicin.

from the EtOAc fraction. Nevertheless, it was suggested that various compounds, except capsaicin metabolites, were present in the EtOAc fraction of *A. oryzae* culture medium. Therefore, in this study, the metabolites in the EtOAc fraction were further investigated. The EtOAc fraction (420 mg) from the culture medium was purified via ODS open-column chromatography with step-wise elution of H₂O/MeOH mixtures, and the 13 fractions (A–M) were grouped via TLC analysis. Fraction B (30% MeOH eluate, 15.3 mg) was subjected to ODS–HPLC (HPLC-2) to give compound **1** (t_R 12.3 min, 11.4 mg). Compounds **2** (t_R 16.8 min, 4.0 mg) and **3** (t_R 17.3 min, 1.8 mg) were isolated from fraction C (30% MeOH eluate, 11.5 mg) using ODS–HPLC (HPLC-3). Compound **4** (11.5 mg) was obtained from fraction E (40% MeOH eluate, 11.5 mg) without further purification. Compounds **5** (t_R 9.8 min, 0.6 mg) and **6** (t_R 17.8 min, 1.0 mg) were isolated from fraction F (40% MeOH eluate, 8.4 mg) using ODS–HPLC (HPLC-4). Compound **7** (t_R 11.2 min, 1.1 mg) was isolated from fraction G (50% MeOH eluate, 4.5 mg) using ODS–HPLC (HPLC-5). Fraction H (50% MeOH eluate, 10.6 mg) was subjected to ODS–HPLC (HPLC-5) to give a mixture of **8** and **9** (t_R 8.7 min, 0.4 mg), **10** (t_R 18.9 min, 2.0 mg), and **11** (t_R 19.9 min, 2.2 mg). The isolation and purification procedures for **1–11** are shown in Fig. 1.

Identification of the isolated compounds The structures of the isolated compounds were determined on the basis of NMR and MS spectroscopic data. Among them, eight known compounds were identified: 5-(hydroxymethyl)-3-furancarboxylic acid (**1**) (15), 3-hydroxypropanoic acid (**2**) (16), 5-(hydroxymethyl)-2-furancarboxylic acid (**3**) (17), 2-(4-hydroxyphenyl)-ethanol (**4**) (18), 4-hydroxybenzoic acid (**5**) (19), vanillic acid (**7**) (20), 3,4-dihydroxybenzoic acid (**8**) (21), and 2-furanol (**9**) (Fig. 2). Compounds **1–5** and **7–9** were identified by comparing the NMR and MS spectroscopic data, as previously reported in the literature. Compounds **6**, **10**, and **11** were identified on the basis of one-dimensional (1D) and two-dimensional (2D) NMR

and MS spectroscopic data.

The ESI-MS (negative) spectrum of **6** showed a pseudomolecular ion peak at m/z 297.1 $[M-H]^-$. This indicates that the molecular weight of **6** is 298. The 1H -NMR (500 MHz, CD₃OD) spectrum of **6** indicated the presence of a double-bond proton at δ 7.25 (H-5) and four methylene protons at δ 5.22 (H-3a), 5.01 (H-3b), 4.81 (H-14a), 3.91 (H-14b), 1.75 (H-7a), 1.28 (H-7b), 2.47 (H-8a), and 1.35 (H-8b) (Table 1). Four methine proton signals at δ 7.25 (H-5a), 1.58 (H-6), 3.73 (H-9a), and 2.11 (H-11) and two methyl proton signals at δ 1.00 (H-12) and 0.93 (H-13) were also observed. The 1H -NMR spectrum was in agreement with the ^{13}C -NMR (125 MHz, CD₃OD) spectrum, which contained a total of 15 carbon signals, including two carbonyl carbons at δ 174.4 (C-1) and 168.6 (C-10) and two quaternary carbons at δ 30.8 (C-4) and 74.1 (C-9) (Table 1). Considering the 1D-NMR and MS results, **6** was suggested to be a bicycle sesquiterpene. The connectivity of **6** was further confirmed via heteronuclear single quantum correlation (HSQC), proton–proton correlation spectroscopy (1H - 1H COSY), and heteronuclear multiple-bond correlation (HMBC) experiments. Correlations between H-9a/H-5a, H-5/H-5a, H-5a/H-6, H-6/H-7, H-7/H-8, H-6/H-11, H-11/H-12, and H-11/H-13 were observed in the 1H - 1H COSY spectrum (Fig. 2, bold lines). Long-range proton–carbon correlations were observed in the HMBC spectrum (Fig. 2, arrows). This indicates that **6** contained 5a,6,7,8,9,9a-hexahydrobenzoxepin-1(3H)-one and isopropyl moieties. In particular, the correlations of H-3 and H-5 to C-10 and H-12 and that of H-13 to C-6 were observed in the HMBC spectrum (Fig. 2, arrows). This indicates that the carboxylic acid group and isopropyl moiety were connected to C-4 and C-6 of 5a,6,7,8,9,9a-hexahydrobenzoxepin-1(3H)-one, respectively. In addition, cross peaks of H-5a and H-7 to C-9, H-14/C-1/C-5, H-5/C-9a, and H-5a/C-1 appeared in the HMBC experiment (Fig. 2). This confirmed that a hydroxymethyl group was located on C-9 of 5a,6,7,8,9,9a-hexahydrobenzoxepin-1(3H)-one. Therefore, the planar structure of compound **6** was determined to be 9-hydroxy-6-

Fig. 2. Structures of the isolated compounds and the important ^1H - ^1H COSY (bold lines) and HMBC (arrows) correlations for **6**, **10**, and **11**.

isopropyl-9-(hydroxymethyl)-1,3,5a,6,7,8,9,9a-octahydro-1-oxobenzoxepine-4-carboxylic acid, a new compound. The ^1H - and ^{13}C -NMR spectra of **6** were similar to those of trichoderonic acid A coupled with a methoxyl group, which was identified from the culture medium of *Trichoderma virens* (22). In the nuclear Overhauser effect (NOE) experiments performed on **6**, the signals of H-11 at δ 2.11 and H-14 at δ 4.81 and 3.91 were enhanced by irradiation of H-5a at δ 2.61, and the signals of H-6 at δ 1.58 were also enhanced by irradiation of H-9a at δ 3.73. Therefore, the structure of compound **6** was determined to (5aS,6R,9S,9aS)-9-hydroxy-6-isopropyl-9-(hydroxymethyl)-1,3,5a,6,7,8,9,9a-octahydro-1-oxobenzoxepine-4-carboxylic acid.

The molecular weight of **10** was determined to be 298 from the pseudomolecular ion peak at m/z 297.1 $[\text{M}-\text{H}]^-$ observed in the ESI-MS (negative) spectrum. The ^1H - and ^{13}C -NMR spectra of **10** showed patterns similar to those of **6**. The ^1H -NMR (CD_3OD , 600 MHz) spectrum of **10** exhibited a double-bond proton signal [δ 6.62 (H-4)], two oxygenated methylene proton signals [δ 4.25 (H-3a), 4.11 (H-3b), 4.46 (H-15a), and 4.06 (H-15b)], two methylene proton signals [δ 1.77 (H-8a), 1.30 (H-8b), 2.01 (H-9a), and 1.67 (H-9b)], four methine proton signals [δ 2.67 (H-5), 2.11 (H-6), 1.34 (H-10), and 1.79 (H-11)], and two methyl proton signals [δ 0.77 (H-12) and 0.97 (H-13)]. The ^1H -NMR spectrum was supported by the ^{13}C -NMR spectrum, which showed a total of 15 carbon signals, including two carbonyl carbon signals at δ 170.3 (C-1) and 179.6 (C-14) and two quaternary carbon signals at δ 135.1 (C-2) and 76.4 (C-7). In addition, the accurate planar structure of **10** was obtained via HSQC, ^1H - ^1H COSY, and HMBC experiments. The important correlations observed in ^1H - ^1H COSY and HMBC experiments are shown in Fig. 2. In particular, the

correlations of H-15 to C-6, C-8, and C-14 were detected in the HMBC spectrum. This indicates that the skeleton structure of **10** was hexahydroisobenzofuran-1(3H)-one. The ^1H - and ^{13}C -NMR spectra of **10** were consistent with those of hydroheptelidic acid, which was identified as a metabolite of *Xylaria* sp. (23). Therefore, **10** was unambiguously identified as hydroheptelidic acid.

The molecular weight of **11** was determined to be 312 from the pseudomolecular ion peak of m/z 311.1 $[\text{M}-\text{H}]^-$ observed in the ESI-MS (negative) spectrum. The ^1H - and ^{13}C -NMR spectra of **11** were also very similar to those of **6**, except for the presence of a methoxyl group [δ 3.39 (3H, s) in the ^1H -NMR spectrum and δ 59.8 in the ^{13}C -NMR spectrum]. On the basis of the MS and 1D-NMR data, **11** was suggested to be trichoderonic acid A. The planar structure of **10** was confirmed via HSQC, ^1H - ^1H COSY, and HMBC experiments. In particular, a correlation between the methoxyl proton signal at δ 3.39 (3H, s) and methylene carbon signal at δ 74.8 (C-14) was detected in the HMBC spectrum (Fig. 2). The ^1H - and ^{13}C -NMR spectra of **11** were consistent with those of trichoderonic acid A. This was identified from the culture medium of *T. virens* (22). Therefore, **11** was unambiguously identified as trichoderonic acid A.

In this study, 11 compounds (**1-11**), except the capsaicin metabolites, were isolated from the culture medium of *A. oryzae* incubated with capsaicin (Fig. 2). A low molecular organic acid (**2**), three furan derivatives (**1**, **3**, and **9**), four phenolics (**4**, **5**, **7**, and **8**), and three sesquiterpenes (**6**, **10**, and **11**) were identified. To the best of our knowledge, this is the first time **6** has been identified in nature. Several studies have implicated that organic acids and phenolics are produced by microorganisms (24-26). Furan aldehyde

compounds such as furfural and 5-hydroxymethylfurfural are present in many foods and determine the flavor properties (27). They are produced via nonenzymatic reactions during the manufacturing of heated foods, such as canned foods and roasted foods (27). Moreover, furan compounds, including furan aldehydes, are metabolized to various furanoic acid derivatives by microorganisms (28). Compound **8** is also found in gochujang fermented by *A. oryzae* (11). These observations suggest that compounds **1-5**, **7**, and **8** identified in this study may be produced by *A. oryzae*; however, some compounds such as the furan derivatives (**1**, **3**, and **9**) may be present in heat-treated media. Previous studies have been reported that 2-(4-hydroxyphenyl)-ethanol (**4**, tyrosol) has diverse biological effects, such as antioxidant, anti-inflammatory, and anti-apoptotic activities (29,30), and *p*-hydroxybenzoic acid derivatives (**5**, **7**, and **8**) also show various bioactivities such as antioxidant, antibacterial, anticancer, and anti-inflammatory activities (31). Therefore, compounds **1-5**, **7**, and **8** are expected to provide information useful for controlling the quality, safety, and biological activity of gochujang.

Three sesquiterpenes (**6**, **10**, and **11**) identified in this study are heptelidic acid derivatives. Compounds **10** and **11** have been found in the culture of fungi, such as *Xylaria* sp. and *T. virens* (22,23), thereby suggesting that **11** is synthesized by methylation of **6**. In addition, heptelidic acid derivatives have been reported to have various biological effects, such as anti-apoptotic (32), anticancer (33), and antimalarial (34) activities. The three heptelidic acid derivatives (**6**, **10**, and **11**) may be produced by *A. oryzae* as secondary metabolites and may act as bioactive compounds in gochujang fermented by *A. oryzae*. Therefore, these heptelidic acid derivatives (**6**, **10**, and **11**) in gochujang fermented by *A. oryzae* for 30 days were quantified via HPLC–ESI–MS using the isolated compounds as external standards. However, the heptelidic acid derivatives (**6**, **10**, and **11**) were not detected in gochujang (data not shown). Possible explanations are as follows. (1) Heptelidic acid derivatives may not be produced by *A. oryzae* during the fermentation of gochujang because it is a high-viscosity paste containing a mixture of primary ingredients such as red hot pepper powder, waxy rice flour, fermented soybean, and koji. (2) Although heptelidic-acid-derivative biosynthesis-related enzymes may be secreted from *A. oryzae* during the fermentation of gochujang, their concentrations and enzymatic activities may be lower than those in PDB culture. Therefore, *A. oryzae* may slowly synthesize heptelidic acid derivatives during the fermentation of gochujang. Further studies on the behavior of heptelidic acid derivatives in gochujang during long-term fermentation by *A. oryzae* are required.

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Disclosure The authors declare no conflict of interest.

References

1. Park JM, Lee SS, Oh HI. Changes in chemical characteristics of traditional *kochujang meju* during fermentation. Korean J. Food Nutr. 8: 184-191 (1995)
2. Choo JJ, Shin HJ. Body-fat suppressive effects of capsaicin through β -adrenergic stimulation in rats fed a high-fat diet. J. Nutr. Health 32: 533-539 (1999)
3. Rhee SH, Kong KR, Jung KO, Park KY. Decreasing effect of *kochujang* on body weight and lipid levels of adipose tissues and serum in rats fed a high-fat diet. J. Korean Soc. Food Sci. Nutr. 32: 882-886 (2003)
4. Park KY, Kong KR, Jung KO, Rhee SH. Inhibitory effect of *kochujang* extracts on the tumor formation. Prev. Nutr. Food Sci. 6: 187-191 (2001)
5. Kwon DY, Hong SM, Ahn IS, Kim YS, Shin DW, Park SM. *Kochujang*, a Korean fermented red pepper plus soybean paste, improves glucose homeostasis in 90% pancreatectomized diabetic rats. Nutrition 25: 790-799 (2009)
6. Kim JY, Park KW, Yang HS, Cho YS, Jeong CH, Shim KH, Yee ST, Seo KI. Anticancer and immuno-activity of methanol extract from onion *kochujang*. Korean J. Food Preserv. 12: 173-178 (2005)
7. Lee KH, Lee MS, Park SO. Studies on the microflora and enzymes influencing on Korean native *kochujang* (red pepper soybean paste) aging. J. Korean Soc. Food Sci. Nutr. 19: 82-92 (1976)
8. Kim YS, Oh BH, Shin DH. Quality characteristics of *kochujang* prepared with different *meju* fermented with *Aspergillus* sp. and *Bacillus subtilis*. Food Sci. Biotechnol. 17: 527-533 (2008)
9. Kozukue N, Han JK, Kozukue E, Lee SJ, Kim JA, Lee KR, Levin CE, Friedman M. Analysis of eight capsaicinoids in peppers and pepper-containing foods by high-performance liquid chromatography and liquid chromatography-mass spectrometry. J. Agr. Food Chem. 53: 9172-9181 (2005)
10. Sacks FM, Lichtenstein A, Horn LV, Harris W, Kris-Etherton P, Winston M. Soy protein, isoflavones, and cardiovascular health: An American heart association science advisory for professionals from the Nutrition Committee. Circulation 113: 1034-1044 (2006)
11. Chung JH, Shin HC, Cho JY, Kang SK, Lee HJ, Shin SC, Park KH, Moon JH. Isolation and structural determination of free radical-scavenging compounds from Korean fermented red pepper paste (*gochujang*). Food Sci. Biotechnol. 18: 463-470 (2009)
12. Shin HC, Cho JY, Lee KH, Ma YK, Lee HY, Park KH, Moon JH. Diterpene glycosides from Korean fermented red pepper paste (*gochujang*) and their origin. Food Chem. 130: 1024-1030 (2011)
13. Cho JY, Lee HJ, Shin HC, Lee JM, Park KH, Moon JH. Behavior of flavonoid glycosides contained in Korean red pepper paste (*gochujang*) during fermentation: Participation of β -glucosidase inhibitor. Food Sci. Biotechnol. 22: 1245-1252 (2013)
14. Lee MJ, Cho JY, Lee YG, Lee HJ, Lim SI, Park SL, Moon JH. Bioconversion of capsaicin by *Aspergillus oryzae*. J. Agr. Food Chem. 63: 6102-6108 (2015)
15. Evidente A, Cristinzio G, Punzo B, Andolfi A, Testa A, Melck D. Flufuran, an antifungal 3,5-disubstituted furan produced by *Aspergillus flavus* LINK. Chem. Biodivers. 6: 328-334 (2009)
16. Schwarz M, Kopcke B, Weber RWS, Sterner O, Anke H. 3-Hydroxypropionic acid as a nematocidal principle in endophytic fungi. Phytochemistry 65: 2239-2245 (2004)
17. Saleem R, Ahmad M, Naz A, Siddiqui H, Ahmad SI, Fizi S. Hypotensive and toxicological study of citric acid and other constituents from *Tagetes patula* roots. Arch. Pharm. Res. 10: 1037-1042 (2004)
18. Wang SJ, Lee HJ, Cho JY, Park KH, Moon JH. Isolation and identification of antioxidants from *Makgeolli*. Korean J. Food Sci. Technol. 44: 14-20 (2012)
19. Rukachaisirikul V, Khamthong N, Sukpondma Y, Phongpachit S, Hutadilok-Towatana N, Graidist P, Sakayaroj J, Kirtikara K. Cyclohexane, diketopiperazine, lactone and phenol derivatives from the sea fan-derived fungi *Nigrospora* sp. PSU-F11 and PSU-F12. Arch. Pharm. Res. 33: 375-380 (2010)
20. Christophoridou S, Dais P, Tseng LH, Spraul M. Separation and identification of phenolic compounds in olive oil by coupling high-performance liquid chromatography with postcolumn solid-phase extraction to nuclear magnetic resonance spectroscopy (LC-SPE-NMR). J. Agr. Food Chem. 53: 4667-4679 (2005)
21. Lee HH, Cho JY, Moon JH, Park KH. Isolation and identification of antioxidative phenolic acids and flavonoid glycosides from *Camellia japonica* flowers. Hort. Environ. Biotechnol. 52: 270-277 (2011)
22. Yamaguchi Y, Manita D, Takeuchi T, Kuramochi K, Kuriyama I, Sugawara F, Yoshida H, Mizushima Y. Novel terpenoids, trichoderonic acids A and B isolated from *Trichoderma virens*, are selective inhibitors of family X DNA polymerases. Biosci. Biotech. Bioch. 74: 798-801 (2010)
23. Yan S, Li S, Wu W, Zhao F, Bao L, Ding R, Gao H, Wen H, Song F, Liu H. Terpenoid and phenolic metabolites from the fungus *Xylaria* sp. associated with termite nests. Chem. Biodivers. 8: 1689-1700 (2011)

24. Tokuyama K, Ohno S, Yoshikawa K, Hirasawa T, Tanaka S, Furusawa C, Shimizu H. Increased 3-hydroxypropionic acid production from glycerol, by modification of central metabolism in *Escherichia coli*. *Microb. Cell Fact.* 13: 64-75 (2014)
25. O'Connor KE, Witholt Bernard, Duetz W. *p*-Hydroxyphenylacetic acid metabolism in *Pseudomonas putida* F6. *J. Bacteriol.* 183: 928-933 (2001)
26. Li K, Frost JW. Synthesis of vanillin from glucose. *J. Am. Chem. Soc.* 120: 10545-10546 (1998)
27. Vranová J, Ciesarová Z. Furan in food – A review. *Czech J. Food Sci.* 27: 1-10 (2009)
28. Wierckx N, Koopman F, Ruijsenaars HJ, de Winde JH. Microbial degradation of furanic compounds: biochemistry, genetics, and impact. *Appl. Microbiol. Biot.* 92: 1095-1105 (2011)
29. Je IG, Kim DS, Kim SW, Lee S, Lee HS, Park EK, Khang D, Kim SH. Tyrosol suppresses allergic inflammation by inhibiting the activation of phosphoinositide 3-kinase in mast cells. *PLoS ONE* 10: e0129829 (2015)
30. Lecci RM, Logrieco A, Leone A. Pro-oxidative action of polyphenols as action mechanism for their proapoptotic activity. *Anti-cancer Agent. Me.* 14: 1363-1375 (2014)
31. Manuja R, Sachdeva S, Jain A, Chaudhary J. A comprehensive review on biological activities of *p*-hydroxybenzoic acid and its derivatives. *Int. J. Pharm. Sci. Rev. Res.* 22: 109-115 (2013)
32. Kim JH, Lee CH. Heptelidic acid, a sesquiterpene lactone, inhibits etoposide-induced apoptosis in human leukemia U937 cells. *J. Microbiol. Biotechnol.* 19: 787-791 (2009)
33. Rahier NJ, Molinier N, Long C, Deshmukh SK, Kate AS, Ranadive P, Verekar SA, Jiotode M, Lavhale RR, Tokdar P, Balakrishnan A, Meignan S, Robichon C, Gomes B, Aussagues Y, Samson A, Sautel F, Bailly C. Anticancer activity of koniginic acid and semisynthetic derivatives. *Bioorgan. Med. Chem.* 23: 3712-3721 (2015)
34. Tanaka Y, Shiomi K, Kamei K, Sugoh-Hagino M, Enomoto Y, Fang F, Yamaguchi Y, Masuma R, Zhang CG, Zhang XW, Omura S. Antimalarial activity of radicicol, heptelidic acid and other fungal metabolites. *J. Antibiot.* 51: 153-160 (1998)