Synthesis of glycosyl ferulate derivatives by amine-promoted glycosylation with regioselective hydrolysis using Novozym 435 and evaluation of their antioxidant properties

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Dedicated to the memory of Dr. Chen-Loung Chen (North Carolina State University, USA)

Key words: amine-promoted glycosylation / enzymatic hydrolysis / regioselectivity / antioxidant property

Abstract

Various glycosyl ferulates were efficiently synthesized from 2,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide (TAGB) with amine by amine-promoted glycosylation without using heavy metal. The resulted acetylated glycosyl ferulates with acetoxyl groups at C-2, C-3 and C-4 were regioselectively deacetylated at C-4 and C-6 positions with Novozym 435. Antioxidant abilities of free ferulic acids and its synthetic glycosyl ferulates were evaluated by inhibitory effect on autoxidation of bulk methyl linoleate as well as their radical scavenging activity. The radical scavenging activity on 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) decreased in the order ferulic acid > sinapinic acid ≈ glycosyl sinapinates ≈ glycosyl ferulates > *p*-coumaric acid > glycosyl *p*-coumarates. In bulk methyl linoleate, the antioxidant activity order against autoxidation was very consistent with the scavenging activity order. The results showed that glycosyl ferulates and sinapinates were effective as well as free carboxylic acid forms.

1. Introduction

It is well established that various glycosyl ferulates are widely occurred in the various plants and have various functions in the plant tissues. Feruloyl-D-glycoside and sinapinoyl-D-glycoside which are contained in the Citrus fruits have anti-oxidation activity. These compounds are reported to have functioned as blood adhesion molecules.[1] It has been reported that *p*-coumaroyl-D-glycosyl ester, one of components in the methanol extract from strawberry has anti-cancer activity although it was only identified and quantified in trace amount.[2] In addition, the other components in the methanol extract from strawberry, methyl and ethyl cinnamate were synthesized in vivo through cinnamoyl glucose esters as intermediates.[3] Although glycosyl ferulates may have similar activities, their syntheses are very rare.[4-6]

In the synthesis of glycosyl esters, catalyst used are mostly heavy metal compounds such as Ag_2CO_3,[7-9] Ag_2O,[10-12] Hg (CN) $_2$,[13-15] $CdCO_3$[16, 17] and $SnCl_4$[18] as Lewis acid. Therefore, not only the synthesis of the glycosyl esters is costly, but also the process is not environmentally friendly. Without using these heavy metal catalysts, the glycosyl esters can be synthesized using the procedure of Fischer by way of esterifing hydroxyl groups of glucose with aglycones, such as alcohols and carboxylic acids. However, in these reactions, the control of stereo-configuration at C-1 of glucose is difficult. Recently, the procedures using microwave,[19] and H_2SO_4-sillica as catalyst[20] are applying for the syntheses of the glycosyl esters, but in these procedures, the difficulty in controlling the stereo-configuration at C-1 of glucose still not completely eliminated.

These lead us to use glycosyl halide for the production of desired glycosyl esters,[21] in which glucoses are converted to the corresponding glycosyl esters with corresponding carboxylic acid using amine to synthesize glycosyl ferulates without using the heavy metal compounds as catalysts and with better control of stereo-configuration at C-1 of glucose. In addition, deacetylation at C-5 and C-6 positions of the resulting acetylated glycosyl esters conducted by Novozym 435 to obtain selective control of stereo-configuration at C-1 of glucose moieties of the corresponding glycosyl esters (Scheme 1).

Ferulic acid is one of the ubiquitous compounds in nature, especially rich as an ester form in rice bran pitch, a byproduct of rice oil production. Application of such industrial waste is important from the environmental point of view. It has been well-known that ferulic acid and *γ*-oryzanol, a mixture of monoesters consisting of ferulic acid and several kinds of triterpene alcohols as cycloartenol and 24-methylenecycloartanol, have an antioxidant activity.[22-24] Such antioxidants are currently expected not only to prevent lipid oxidation in food but also to prevent free-radical-induced diseases such as cancer and atherosclerosis or aging caused by oxidative tissue degeneration.[25] Therefore, there is a possibility of developing an application of rice bran pitch for human health.

In general, the inhibitory effect of antioxidants on lipid oxidation is influenced by the physicochemical state of the lipid substance, and several evaluation systems using different physical conditions are required for better understanding of antioxidant abilities.[26] Although ferulic acid and its glycosyl ester have been recognized as antioxidants, there are few reports on systematic evaluation of the antioxidant properties of ferulic acid and its derivatives in different conditions.

In this paper, we described the antioxidant activities of ferulic acid and its synthetic glycosyl ferulates, as well as some ferulic acid related compounds such as *p*-coumaric acid, sinapinic acid, and these glycosides. Their antioxidant activity was evaluated on the basis of their inhibitory effects on the autoxidation of methyl linoleate in bulk system and the radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$).

2. Experimental

2.1. General

IR spectra were measured on an FT-IR 460plus spectrometer (JASCO Corp., Tokyo, Japan). ^1H and ^{13}C NMR spectra were measured on a JNM-ECA-500 spectrometer (JEOL, Tokyo, Japan) at 500 and 126 MHz, respectively. Deuterated solvents were used as internal standards. Structure determination of all compounds was performed by the use of COSY, HMQC, and HMBC NMR techniques. The high resolution ESI-MS spectra of 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl ferulate (**1c**), 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl sinapinate (**1d**),

2,3-di-*O*-acetyl-*β*-D-glucopyranosyl cinnamate (**2a**), 2,3-di-*O*-acetyl-*β*-D-glucopyranosyl ferulate (**2c**) and 2,3-di-*O*-acetyl-*β*-D-glucopyranosyl sinapinate (**2d**) were measured on a JMS-T100LC mass spectrometer (JEOL, Japan) equipped with UV detector (254 nm) and Cadenza CD-C-18 (4.6 mmID × 150 mm, Imtakt) column. The LC gradient proceeded from 70% acetonitrile and 30% water acidified with 0.1% trifluoroacetic acid to 20% acetonitrile and 80% acidic water at a flow rate of 1.0 mL/min. The high resolution APCI-MS spectrum of 2,3-di-*O*-acetyl-*β*-D-glucopyranosyl *p*-coumarate (**2b**) was measured on a JMS-T100LC mass spectrometer (JEOL, Japan) equipped with UV detector (254 nm) and Synergi MAX-RP 80A (2.0 mmID × 150 mm, Phenomenex) column. The LC gradient proceeded from 60% acetonitrile and 40% water acidified with 0.1% trifluoroacetic acid to 30% acetonitrile and 70% acidic water at a flow rate of 0.2 mL/min. Absorbance spectra were measured using an UVmini-2400 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Flash column chromatography was performed using silica gel FL60D (Fuji Silysia Chemical Ltd., Aichi, Japan). Thin layer chromatography was performed with silica gel F-254 on aluminum plate (Merck Ltd., Darmstadt, Germany). Solvents were dried following standard methods. D-Glucose was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). *Trans*-cinnamic acid, *p*-coumaric acid and sinapinic acid were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Ferulic acid was purchased from Tsuno Co. Ltd. (Wakayama, Japan). Novozym 435 (immobilized lipase from *Candia Antarctica*) was a gift from Novozymes A/S (Paraná, Brazil). All other materials were commercially obtained.

2.2. Glucosylation of ferulic acids

2.2.1. 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranosyl cinnamate (1a).

A solution of 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide (411 mg, 1.0 mmol), *trans*-cinnamic acid (296 mg, 2.0 mmol) and *i*-Pr_2NEt (388 mg, 3.0 mmol) in CH_3CN (5 mL) was stirred for 24 hours at room temperature under Ar atmosphere, and 1 g 4Å molecular sieves was used for water binding. Progress of the reaction was monitored by thin layer chromatography. On the completion of the reaction, the reaction mixture was filtered to remove molecular sieves, and the solvent was evaporated under vacuum from the reaction mixture, and EtOAc was added to the residue. The resulting solution was then neutralized with aqueous $NaHCO_3$, and extracted with EtOAc, followed by wash with brine. The combined extracts were dried over anhydrous $MgSO_4$, and the solvent was removed by evaporating under vacuum. The crude product was purified by flash column chromatography (hexane-EtOAc, 3:1) to give **1a** (244mg, 78%) as a white solid. R_f = 0.50 (hexane-EtOAc, 1:1); $[\alpha]^{20}_D$ -17.7 (*c* 1.0, THF) [lit.$[\alpha]^{20}_D$= -32 (*c* 0.75, EtOH)[27]]; IR (KBr): 3011, 1745, 1135, 943 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.06 (m, 12H, C<u>H</u>$_3$×4 in OAc at C-2, C-3, C-4 and C-6), 3.91 (ddd, *J* = 9.2, 4.6, 2.3 Hz, 1H, H-5), 4.13 (dd, *J* = 12.6, 2.3 Hz, 1H, H-6a), 4.32 (dd, *J* = 12.6, 4.6 Hz, 1H, H-6b), 5.18 (t, *J* = 9.2 Hz, 1H, H-4), 5.22 (t, *J* = 9.2 Hz, 1H, H-2), 5.28 (t, *J* = 9.2 Hz, 1H, H-3), 5.86 (d, *J* = 9.2Hz, 1H, H-1), 6.42 (d, *J* = 16.0 Hz, 1H, -C(=O)C<u>H</u>=CH-), 7.41 (m, 3H, Ph), 7.54 (m, 2H, Ph), 7.76 (d, *J* = 16.0 Hz, 1H, -C(=O)CH=C<u>H</u>-); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 20.6 (-<u>C</u>H$_3$ in OAc at C-2 and C-4), 20.7 (-<u>C</u>H$_3$ in OAc at C-3 and C-6), 61.5 (C-6), 67.8 (C-4), 70.3 (C-2), 72.7 (C-3, C-5), 91.9 (C-1), 116.2 (-C(=O)<u>C</u>H=CH-), 128.4 (Ph), 129.0 (Ph), 130.9 (Ph), 133.8 (Ph), 147.5 (-C(=O)CH=<u>C</u>H-), 164.6 (-<u>C</u>(=O)CH=CH-), 169.3(-<u>C</u>=O in OAc at C-2), 169.4 (-<u>C</u>=O in OAc at C-3), 170.0 (-<u>C</u>=O in OAc at C-4) 170.6 (-<u>C</u>=O in OAc at C-6).

2.2.2. 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranosyl *p*-coumarate (1b).

This compound was synthesized according to procedure described for **1a**. Instead of *trans*-cinnamic acid, *p*-coumaric acid (328 mg, 2.0 mmol) was glycosylated with TAGB (411 mg, 1.0 mmol) to give **1b** (401 mg, 81%) as a white solid. R_f = 0.45 (hexane-EtOAc, 1:1); $[\alpha]^{20}_D$ -2.9 (*c* 1.0, THF) (lit.[α] Negative specific rotation, $CHCl_3$[28]); IR (KBr): 3354, 3024, 1756, 1715, 1629, 1603, 1588, 1516, 1231, 1081 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.06 (m, 12H, C<u>H</u>$_3$×4 in OAc at C-2, C-3, C-4, C-6), 3.92 (ddd, *J* = 9.2, 4.6, 2.3 Hz, 1H, H-5), 4.16 (dd, *J* = 12.6, 2.3 Hz, 1H, H-6a), 4.33 (dd, *J* = 12.6, 4.6 Hz, 1H, H-6b), 5.19 (t, *J* = 9.2 Hz, 1H, H-4), 5.22 (t, *J* = 9.2 Hz, 1H, H-2), 5.28 (t, *J* = 9.2 Hz, 1H, H-3), 5.86 (d, *J* = 9.2 Hz, 1H, H-1), 6.23 (d, *J* = 15.5 Hz, 1H, -C(=O)C<u>H</u>=CH-), 6.86 (d, *J* = 8.6 Hz, 2H, Ph), 7.41 (d, *J* = 8.6 Hz, 2H, Ph), 7.68 (d, *J* = 16.0 Hz, 1H, -C(=O)CH=C<u>H</u>-); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 20.6 (<u>C</u>H$_3$ in OAc at C-2 and C-4), 20.7 (<u>C</u>H$_3$ in OAc at C-3 and C-6), 61.5 (C-6), 67.8 (C-4), 70.3 (C-2), 72.7 (C-5), 72.8 (C-3), 91.8 (C-1), 113.3 (-C(=O)<u>C</u>H=CH-), 116.0 (Ph), 126.5 (Ph), 130.4 (Ph), 147.3 (-C(=O)CH=<u>C</u>H-), 158.6(Ph), 165.1(-<u>C</u>(=O)CH=CH-), 169.5(-<u>C</u>=O in OAc at C-2), 169.6 (-<u>C</u>=O in OAc at C-3), 170.2 (-<u>C</u>=O in OAc at C-4), 170.8 (-<u>C</u>=O in OAc at C-6).

2.2.3. 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranosyl ferulate (1c).

This compound was synthesized according to procedure described for **1a**. Instead of *trans*-cinnamic acid, ferulic acid (388 mg, 2.0 mmol) was glycosylated with TAGB (411 mg, 1.0 mmol) to give **1c** (388 mg, 74%) as a white solid. R_f = 0.34 (hexane-EtOAc, 1:1); $[\alpha]^{20}_D$ -52.7 (*c* 1.0, THF); IR (KBr): 3382, 2969, 1754, 1638, 1588, 1515, 1372, 1233, 1098 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.05 (m, 12H, C<u>H</u>$_3$×4 in OAc at C-2, C-3, C-4, C-6), 3.90 (ddd, *J* = 9.2, 4.6, 2.3 Hz, 1H, H-5), 3.94 (s, 3H, C<u>H</u>$_3$ in OMe at Ph), 4.13 (dd, *J* = 12.6, 2.3 Hz, 1H, H-6a), 4.32 (dd, *J* = 12.6, 4.6 Hz, 1H, H-6b), 5.18 (t, *J* = 9.2 Hz, 1H, H-4), 5.22 (t, *J* = 9.2 Hz, 1H, H-2), 5.28 (t, *J* = 9.2 Hz, 1H, H-3), 5.86 (d, *J* = 9.2 Hz, 1H, H-1), 5.91 (s, 1H, OH), 6.26 (d, *J* = 16.0 Hz, 1H, -C(=O)C<u>H</u>=CH-), 6.93 (d, *J* = 8.6 Hz, 1H, Ph), 7.04 (d, *J* = 1.7 Hz, 1H, Ph), 7.09 (dd, *J* = 8.3, 2.0 Hz, 1H, Ph), 7.69 (d, *J* = 16.0 Hz, 1H, -C(=O)CH=C<u>H</u>-); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 20.6 (<u>C</u>H$_3$ in OAc), 56.0 (O<u>C</u>H$_3$ at Ph), 61.5 (C-6), 67.9 (C-4), 70.3 (C-2), 72.7 (C-3, C-5), 91.9 (C-1), 109.6 (Ph), 113.4 (-C(=O)<u>C</u>H=CH-), 114.8 (Ph), 123.9 (Ph), 126.5 (Ph), 146.8 (Ph), 147.6 (-C(=O)CH=<u>C</u>H-), 148.6 (Ph), 164.9 (-<u>C</u>(=O)CH=CH-), 169.4 (2-<u>C</u>=O in OAc at C-3 and C-4), 170.0 (-<u>C</u>=O in OAc at C-6), 170.6 (-<u>C</u>=O in OAc at C-2). ESI-MS: *m*/*z* [M + NH_4]$^+$ calcd for $C_{24}H_{32}NO_{13}$: 542.1873; found: 542.1868.

2.2.4. 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranosyl sinapinate (1d).

This compound was synthesized according to procedure described for **1a**. Instead of *trans*-cinnamic acid, sinapinic acid (448 mg, 2.0 mmol) was glycosylated with TAGB (411 mg, 1.0 mmol) to give **1d** (405 mg, 73%) as a orange solid. R_f = 0.24 (hexane-EtOAc, 1:1); $[\alpha]^{20}_D$ -6.8 (*c* 1.0, THF); IR (KBr): 3471, 2943, 1751, 1545, 1223, 1070 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.07 (m, 12H, C<u>H</u>$_3$×4 in OAc), 3.91 (ddd, *J* = 9.2, 4.6, 2.3 Hz, 1H, H-5), 3.93 (s, 6H, O<u>C</u>H$_3$ at Ph), 4.14 (dd, *J* = 12.6, 2.3 Hz, 1H, H-6a), 4.32 (dd, *J* =12.6, 4.6 Hz, 1H, H-6b), 5.18 (t, *J* = 9.2 Hz, 1H, H-4), 5.22 (t, *J* = 9.2 Hz, 1H, H-2), 5.28 (t, *J* = 9.2 Hz, 1H, H-3), 5.82 (s, 1H, OH), 5.87 (d, *J* = 9.2 Hz, 1H, H-1), 6.28 (d, *J* = 16.0 Hz, 1H, -C(=O)C<u>H</u>=CH-), 6.79 (s, 2H, Ph), 7.67 (d, *J* = 16.0 Hz, 1H, -C(=O)CH=C<u>H</u>-); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 20.5 (<u>C</u>H$_3$×2 in OAc), 20.6 (<u>C</u>H$_3$ in OAc), 56.3 (<u>C</u>H$_3$

in OCH_3 at Ph), 61.5 (C-6), 67.8 (C-4), 70.2 (C-2), 72.6 (C-5), 72.7 (C-3), 91.7 (C-1), 105.4 (Ph), 113.7 (-C(=O)CH=CH-), 125.3 (Ph), 137.8 (Ph), 147.2 (Ph), 147.7 (-C(=O)CH=CH-), 164.8 (-C(=O)CH=CH-), 169.4 (2-C=O in OAc at C-3 and C-4), 170.0 (-C=O in OAc at C-6), 170.6 (-C=O in OAc at C-2). ESI-MS: *m*/*z* $[M + NH_4]^+$ calcd for $C_{25}H_{34}NO_{14}$: 572.1981; found: 572.1979.

2.3. Regioselective deacetylation with Novozym 435

2.3.1. 2,3-Di-*O*-acetyl-*β*-D-glucopyranosyl cinnamate (2a).

A solution of 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl cinnamate (**1a**) (478 mg, 1.0 mmol), MeOH (256 mg, 8.0 mmol) and Novozym 435 (0.8 g) in *tert*-butyl methyl ether (10 mL) was stirred for 24 hours at 50°C. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, Novozym 435 was filtered off the reaction mixture, and the solvent was then removed under vacuum. The residue was purified with flash chromatography ($CHCl_3$-MeOH, 20:1) to give **2a** (386 mg, 98%) as a white solid. R_f = 0.42 ($CHCl_3$-MeOH, 9:1); $[\alpha]^{20}_D$ -49.4 (*c* 1.0, THF); IR (KBr): 3446, 2935, 1751, 1635, 1244, 1074, 1034 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.92 (s, 3H, C<u>H</u>$_3$ in OAc at C-2), 2.00 (s, 3H, C<u>H</u>$_3$ in OAc at C-3), 2.98 (br, 1H, OH at C-6), 3.53 (m, 1H, H-5), 3.66 (m, 2H, H-4, H-6a), 3.83 (d, *J* = 10.7 Hz, 1H, H-6b), 3.86 (br, 1H, OH at C-4), 4.98 (t, *J* = 9.2 Hz, 1H, H-2), 5.13 (t, *J* = 9.2 Hz, 1H, H-3), 5.80 (d, *J* = 9.2 Hz, 1H, H-1), 6.22 (d, *J* = 16.5 Hz, 1H, -C(=O)C<u>H</u>=CH-), 6.74 (m, 3H, Ph), 7.39 (d, *J* = 8.9 Hz, 2H, Ph), 7.60 (d, *J* = 16.0 Hz, 1H, -C(=O)CH=C<u>H</u>-); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 20.8 (<u>C</u>H$_3$ in OAc at C-2), 21.0 (<u>C</u>H$_3$ in OAc at C-3), 61.8 (C-6), 68.9 (C-4), 70.7 (C-2), 75.7 (C-3), 76.7 (C-5), 92.3 (C-1), 116.4 (-C(=O)<u>C</u>H=CH-), 128.6 (Ph), 129.1(Ph), 131.1 (Ph), 134.0 (Ph), 147.7 (-C(=O)CH=<u>C</u>H-), 165.2 (-<u>C</u>(=O)CH=CH-), 170.0 (-<u>C</u>=O in OAc at C-2), 171.5 (-<u>C</u>=O in OAc at C-3). ESI-MS: *m*/*z* $[M + NH_4]^+$ calcd for $C_{19}H_{26}NO_9$: 412.1602: found: 412.1608.

2.3.2. 2,3-Di-*O*-acetyl-*β*-D-glucopyranosyl *p*-coumarate (2b).

This compound was synthesized according to procedure described for **2a**. 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl *p*-coumarate (**1b**) (468 mg, 1.0 mmol) was hydrolyzed with Novozym 435 to give **2b** (267 mg, 65%) as a white solid. R_f = 0.42 ($CHCl_3$-MeOH, 9:1); $[\alpha]^{20}_D$ -49.7 (*c* 1.0, THF); IR (KBr): 3351, 2921, 1721, 1604, 1246, 1067, 1036 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 1.92 (s, 3H, C<u>H</u>$_3$ in OAc at C-2), 2.00 (s, 3H, C<u>H</u>$_3$ in OAc at C-3), 3.53 (m, 1H, H-5), 3.66 (m, 2H, H-4, H-6a), 3.83 (d, *J* = 10.7 Hz, 1H, H-6b), 4.75 (t, *J* = 5.7 Hz, 1H, OH at C-6), 4.98 (t, *J* = 9.2 Hz, 1H, H-2), 5.13 (t, *J* = 9.2 Hz, 1H, H-3), 5.56 (d, *J* = 5.7 Hz, 1H, OH at C-4), 5.80 (d, *J* = 9.2 Hz, 1H, H-1), 6.22 (d, *J* = 16.5 Hz, 1H, -C(=O)C<u>H</u>=CH-), 6.74 (d, *J* =7.6 Hz, 2H, Ph), 7.39 (d, *J* = 8.9 Hz, 2H, Ph), 7.60 (d, *J* = 16.0 Hz, 1H, -C(=O)C<u>H</u>=C<u>H</u>-); ^{13}C NMR (126 MHz, CD_3OD): δ = 20.6 (<u>C</u>H$_3$ in OAc at C-2), 20.8 (<u>C</u>H$_3$ in OAc at C-3), 61.8 (C-6), 68.9 (C-4), 72.4 (C-2), 76.7 (C-3), 78.5 (C-5), 93.3 (C-1), 113.6 (-C(=O)<u>C</u>H=CH-), 116.9 (Ph), 126.8 (Ph), 131.5 (Ph), 148.5 (-C(=O)CH=<u>C</u>H-), 161.6 (Ph), 166.8 (-<u>C</u>(=O)CH=CH-), 171.3(-<u>C</u>=O in OAc at C-2), 172.1 (-<u>C</u>=O in OAc at C-3). APCI-MS: *m*/*z* $[M + Na]^+$ calcd for $C_{19}H_{22}NaO_{10}$: 433.1116; found: 433.1111.

2.3.3. 2,3 -Di-*O*-acetyl-*β*-D-glucopyranosyl ferulate (2c).

This compound was synthesized according to procedure described for **2a**. 2,3,4,6-di-*O*-acetyl-*β*-D-glucopyranosyl ferulate (**1c**) (361 mg, 1.0 mmol) was hydrolyzed with Novozym 435 to give **2c** (361 mg, 82%) as a white solid. R_f = 0.32 ($CHCl_3$-MeOH, 9:1); $[\alpha]^{20}_D$ -44.8 (*c* 1.0, THF); IR (KBr) 3464, 3370, 1740, 1248, 1078 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.95 (s, 3H, C<u>H</u>$_3$ in OAc at C-2), 2.01 (s, 3H, C<u>H</u>$_3$ in OAc at C-3), 3.34 (m, 1H, H-5), 3.55 (m, 3H, H-4, H-5, H-6a), 3.68 (dd, *J* = 11.2, 3.7 Hz, 1H, H-6b), 3.82 (s, 3H, C<u>H</u>$_3$ in OMe at Ph), 4.66 (s, 1H, OH at C-6), 4.86 (t, *J* = 9.2 Hz, 1H, H-2), 5.12 (t, *J* = 9.2 Hz, 1H, H-3), 5.57 (s, 1H, OH at C-4), 5.90 (d, *J* = 9.2 Hz, 1H, H-1), 6.46 (d, *J* = 16.0 Hz, 1H, -C(=O)C<u>H</u>=CH-), 6.80 (d, *J* = 8.0 Hz, 1H, Ph), 7.14 (dd, *J* = 8.3, 2.0 Hz, 1H, Ph), 7.35 (s, 1H, Ph), 7.60 (d, *J* = 16.0 Hz, 1H, -C(=O)CH=C<u>H</u>-), 9.74 (br, 1H, PhOH); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 20.4 (<u>C</u>H$_3$ in OAc at C-2), 20.7 (<u>C</u>H$_3$ in OAc at C-3), 55.7 (<u>C</u>H$_3$ in OCH$_3$ at Ph), 60.0 (C-6), 67.9 (C-5), 70.9 (C-2), 74.9 (C-3), 77.3 (C-4), 91.3 (C-1), 111.3 (Ph), 112.8 (-C(=O)<u>C</u>H=CH-), 115.5 (Ph), 123.9 (Ph), 125.2 (Ph), 147.3 (-C(=O)CH=<u>C</u>H-), 147.9 (Ph), 150.0 (Ph), 164.8 (-<u>C</u>(=O)CH=CH-), 169.3 (-<u>C</u>=O in OAc at C-2), 169.7 (-<u>C</u>=O in OAc at C-3). ESI-MS: *m*/*z* [M + NH^4]$^+$ calcd for $C_{20}H_{28}NO_{11}$: 458.1662; found: 458.1668.

2.3.4. 2,3-Di-*O*-acetyl-*β*-D-glucopyranosyl sinapinate (2d).

This compound was synthesized according to procedure described for **2a**. 2,3,4,6-di-*O*-acetyl-*β*-D-glucopyranosyl sinapate (**1d**) (528 mg, 1.0 mmol) was hydrolyzed with Novozym 435 to give **3d** (423 mg, 90%) as a white solid. R_f = 0.43 ($CHCl_3$-MeOH, 9:1); $[\alpha]^{20}_D$ -39.6 (*c* 1.0, THF); IR (KBr): 3446, 2997, 1740, 1412, 1135, 942 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.95 (s, 3H, C<u>H</u>$_3$ in OAc at C-2), 2.00 (s, 3H, C<u>H</u>$_3$ in OAc at C-3), 3.54 (m, 3H, H-4, H-5, H-6a) 3.68(m, 1H, H-6b), 3.80 (s, 6H, C<u>H</u>$_3$×2 in OMe at Ph), 4.75 (t, *J* = 5.7 Hz, 1H, OH at C-6), 4.86 (t, *J* = 9.2 Hz, 1H, H-2), 5.11 (t, *J* = 9.2 Hz, 1H, H-3), 5.56 (d, *J* = 5.7 Hz, 1H, OH at C-4), 5.91 (d, *J* = 9.2 Hz, 1H, H-1), 6.52 (d, *J* = 16.0 Hz, 1H, -C(=O)C<u>H</u>=CH-), 7.06 (s, 2H, Ph), 7.61 (d, *J* = 16.0 Hz, 1H, -C(=O)CH=C<u>H</u>-), 9.08 (br, 1H, Ph<u>OH</u>); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 20.2(<u>C</u>H$_3$ in OAc at C-2), 20.6 (<u>C</u>H$_3$ in OAc at C-3), 56.1 (<u>C</u>H$_3$ in OCH$_3$ at Ph), 60.0 (C-6), 67.1 (C-4), 70.9 (C-2), 75.0 (C-3), 77.3 (C-5), 96.1 (C-1), 106.7 (Ph), 113.2 (-C(=O)<u>C</u>H=CH-), 124.0 (Ph), 138.9 (Ph), 147.4 (-C(=O)CH=<u>C</u>H-), 148.0 (Ph), 164.7(-<u>C</u>(=O)CH=CH-), 169.0 (-<u>C</u>=O in OAc at C-2), 169.6(-<u>C</u>=O in OAc at C-3). ESI-MS: *m*/*z* [M + NH_4]$^+$ calcd for $C_{21}H_{30}NO_{12}$: 488.1777; found: 488.1768.

2.4. Evaluation of scavenging effect on DPPH˙ radicals.

The antioxidant potential of glycosyl ferulates was estimated by measuring their free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH˙) as free radical according to modification of the method of Silva et al.[29] The reaction mixture (10 mL) comprised of freshly made 0.15 mM DPPH˙ in ethanol (7000 *μ*L), different concentrations of each glycosyl ferulates (1, 5, and 10 *μ*mol) in 300 *μ*L DMSO, and Tris-HCl buffer (pH 7.4, 100 mM). The reaction mixture was kept for 30 min in a water bath at 25°C under dark and optical density was measured at 517 nm. DPPH˙ has an unpaired electron, which gives purple color, and when this electron is balanced, the color is lost. The compounds which can give an electron to the DPPH˙ can bleach the color. The scavenging activity of tested compound was measured as the decrease in absorbance of the DPPH˙ solution without test compounds. All analyses were carried out in triplicate.

2.5. Evaluation of the inhibitory effect on autoxidation of methyl linoleate.

To 1 g of methyl linoleate, 25 μL of the acetone solution of the test compound was mixed in a 50 mL vial, and the mixture was agitated under ultrasonic wave for 30 s. A 25 μL aliquot of acetone without sample was added for control. After purging the acetone with nitrogen, the mixture was placed in an oven at 40°C in dark. Final concentration of each sample was 0.05 μmol/g oil. An aliquot of oil sample was at 234 nm with a UVmini-2400 UV-Vis spectrophotometer every 24 h at 20°C. All tests were run in triplicate.

3. Results and discussion

3.1. Glucosylation of ferulic acids

Glucosylated carboxyl acids derived from cinnamic acid, such as *trans*-cinnamic acid, *p*-coumaric acid, ferulic acid and sinapinic acid, were achieved from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (TAGB) via S_N2 reaction of the bromide leaving group to the corresponding 2,3,4,6-tetra-*O*-acetyl-α-D-glycopyranosyl carboxylate with high yield (more that 70%, except for **1b**), respectively. *i*-Pr_2NEt and Et_3N were used as a Brønsted base to deprotonate the carboxylic acid making it more nucleophilic. In the case of **1b** using *i*-Pr_2NEt as a base, the yield is lower than that of other glycosyl esters (see Table 1, Entry 2). Precipitate was observed only in the preparation of **1b**. It seemed that carboxylic acid and *i*-Pr_2NEt made it the salt and this lead to the low yield. Therefore, Et_3N was used instead of *i*-Pr_2NEt, and the salt was not observed. The yield of **1b** increased to as high as 81%.

3.2. Regioselective deacetylation with Novozym 435

In order to deacetylation of acetoxyl groups at C-2, C-3, C-4, and C-6, inorganic bases were first used to hydrolysis. However, the acetoxyl group at C-1 was also unexpectedly undergoing hydrolysis. Therefore, we had made attention in lipase that has the capability for regioselective deacetylation. Among lipase, Novozym 435 has wide substrate specificity, and well established properties in stereo- and regioselective activities in hydrolysis of ester groups. Therefore, it could be hydrolyzed acetoxyl groups at C-2, C-3, C-4, and C-6 of the tetra-*O*-acetyl glycosyl esters without hydrolyzing the ester group at C-1. In hydrolysis of esters with Novozym 435, ethyl ether is in general used as solvent. However, in hydrolysis of the tetra-*O*-acetyl glycosyl esters with Novozym 435, *t*-BuOMe was used as solvent. In addition, the optimal reaction temperature is 50°C where Novozym 435 has the maximum activity. When the tetra-*O*-acetyl glycosyl esters **1b** and **1c** were used as substrates, they were not completely soluble in *t*-BuOMe, as a consequence a mixture of *t*-BuOMe/benzene (3:1) was used to hydrolysis of the substrates. As the results, the 24 hours reaction proceeded normally for all substrates. However, all of the products were glycosyl esters **2**, in which only acetoxyl groups at position C-4 and C-6 were hydrolyzed (Table 2). In addition, the reaction time was extended to 24 hours, and the reaction was not preceded any more and yields of the products. From these results, it has been identified that the Novozym 435 has selectively hydrolyzed acetoxyl groups at position C-4 and C-6 in glycosyl esters **1**. Although glycosyl cinnamate **1a** was consumed completely during the enzymatic hydrolysis, substrates **1b** and **1d** were detected in the reaction mixture. Thus, the regioselectivity of Novozym 435 for substrate specificity, the

glycosyl ester **1a** is the highest (Table 2, Entry 1), and the enzyme showed least affinity to **1b** (Table 2, Entry 2). The active site of *Candida antarctica* lipase B (Novozym 435) was reported.[30] Active site of lipase, catalytic triad, is generally formed by three amino acids. These are serine, which is active center, histidine and aspartate. The active site of *Candida antarctica* lipase B is composed by Ser105, His187 and Asp224. The reaction mechanism of lipase-catalyzed hydrolysis of esters was reported.[31] The reaction of hydrolysis starts with the formation of tetrahedral intermediate with alcohol, where ester as a substrate is covalently linked to side-chain oxygen of catalytic serine. The crucial hydrogen bond from H of catalytic histidine to serine O and the oxygen of alcohol moiety are formed. Transfer of H to the oxygen of the alcohol moiety splits away the alcohol. It assumed that the hydrolyzed glycosyl esters (**2a-d**) are produced by this mechanism. Steric hindrance of the acetoxyl group at C-6 was smaller than that of other acetoxyl groups and feruloyloxyl group because of the primary alcohol at glucose C-6 position. Steric hindrance of the acetoxyl group at C-4 was also smaller compared with that at C-2 and C-3 for the methylene group at C-6. For this reason, Novozym 435 preferentially incorporated the acetoxyl group at C-4 and C-6 into its active site, and hydrolyzed these acetoxyl groups into glycosyl esters **2**. Naoshima *et al.* reported that the C-O distance between the oxygen of serine and the carbonyl carbon of substrate ester affected lipase selectivity.[32] Lipase preferentially hydrolyzed substrate esters which had the short C-O distance. It seemed that the small steric hindrance had come easily close to the oxygen of serine at active site, and the acetoxyl groups at C-4 and C-6 hydrolyzed. The progress of Novozym 435-catalyzed hydrolysis was monitored by TLC, and only hydrolyzed glycosyl esters **2** except unreacted **1** was observed. This showed that Novozym 435 specifically hydrolyzed the acetoxyl groups at C-4 and C-6. Kazlauskas *et al*. suggested that the relationship between the structure of active site and substrate to enantioselectivity.[33] An empirical rule, which is based only on the presence of steric factors in substrate molecules, was proposed to predict the enantioselectivity toward secondary alcohols and their esters displayed by lipase. Recently, the relationship between structure of active site and substrate and enantioselectivity of lipase is reported.[34-37] Lemke *et al*. investigated particularly about the enantioselectivity of *Pseudomonas cepacia* lipase using 69 kinds of substrate.[38] The kinetic resolution of 3-(aryloxy)propan-2-ol derivatives by transesterification with vinyl acetate in organic solvents in the presence of *Pseudomonas cepacia* lipase was subjected. This lipase showed high *E* value (>100) to the substrates which has acyloxy group such as *n*-pentanoate, *n*-nonanoate, and *n*-pentadecanoate. In contrast, it expressed low *E* value, about 30, to the similar substrate possessed *n*-hexanoate, *n*-octanoate, and *n*-heptadecanoate. The slight difference of chain length has a great effect on the enantioselectivity of lipase. Furthermore, Lemke *et al*. concluded that the enantioselectivity of lipase was determined not by volume of active site but by shape. In this paper, Novozym 435 exhibited not enantioselectivity but regioselectivity for hydrolysis of acetylated glycosyl esters. However, it seemed that in this case in which lipase recognized not the volume but the shape of substrate applied to Novozym 435-catalyzed regioselective hydrolysis. Although all acetoxyl groups at C-2, C-3, C-4 and C-6 were the same structure and shape, each steric hindrance around every acetoxyl group was different. It assumed that Novozym 435 recognized the shape of substrate, and specifically hydrolyzed only acetoxyl groups at C-4 and C-6.

3.3. Radical scavenging of ferulic acids and its glycosyl esters

The present study demonstrates the antioxidant properties of ferulic acids and glycosyl ferulate derivatives synthesize by evaluation of the DPPH· radical scavenging activity and the inhibitory effect on autoxidation of methyl linoleate in bulk phase. **Figure 1** shows the DPPH· radical scavenging activity of ferulic acids and the corresponding glycosyl ferulates. Scavenging ability of all tested compounds increased with concentration in the range of 0.1 to 1.0 μmol/mL. Ferulic acid had the best radical scavenging activity compared with other tested compounds, with the ability about 90% in all concentrations. The activity decreased in the order ferulic acid > sinapinic acid ≈ glycosyl sinapinates (**1d** ≈ **2d**) > glycosyl ferulates (**1c** < **2c**) > *p*-coumaric acid > glycosyl *p*-coumarates (**1b** < **2b**). The activity of sinapinic acid was somewhat lower than that of ferulic acid. These data are in disagreement with results reported by Pekkarinen *et al.* and Kylli *et al.*, who measured the DPPH· radical scavenging of ferulic acids.[39,40] These discrepancies may be due to experimental differences, such as antioxidant concentration and a composition of medium. Tested glycosyl ferurates except glycosyl sinapinates (**1d** and **2d**) showed lower activity compared with free ferulic acids. With sinapinic acids no great difference was seen. This may be explained by the great antioxidant potency of sinapinic acid and its glycosyl esters compared to other ferulic acids. An increase in concentration of antioxidant in the presence of ferulic acid led to no change in its DPPH· radical scavenging activity, while the activity of glycosyl ferulates (**1b** and **2b**) increased with concentration. A possible explanation for the concentration dependent might be a slight difference in the solubility in the test solvent. Glycosyl ferulates possessed acetoxyl and/or hydroxyl groups, and these high polar groups raise the solubility in the tested medium. It seemed that the lower polarity of free ferulic acid with in ferulic acid derivatives might result in higher effectiveness on DPPH· radical scavenging effect in good agreement with "polar paradox", according to which polar antioxidants are more efficient in an apolar medium, and conversely, apolar antioxidants are more efficient in a polar medium.[41] Free *p*-coumaric acid and its glycosyl esters (**1b** and **2b**) showed lower scavenging activity compared with ferulic and sinapinic acid derivatives. The scavenging ability of hydroxycinnamic acids against DPPH· radical was dependent on the number of hydroxyl groups on the benzene ring and ortho substitution with the electron donor methoxy group which increases the stability of the phenoxy radical.[39,42] Therefore, the ability of ferulic and sinapinic acid derivarives was higher than that of *p*-coumaric acid derivatives. In *p*-coumaric acid derivarives, glycosyl *p*-coumarates (**1b** and **2b**) exhibited lower ability compared with free *p*-coumaric acid, and there was no great difference among glycosyl *p*-coumarates. Pekkarinen *et al.* explained the low antioxidant activity of 2,3-dihydroxybenzoic acid.[40] 2,3-Dihydroxybenzoic acid forms an intramolecular hydrogen bond between carbonyl oxygen and hydroxyl group hydrogen atom, which would explain a low activity in DPPH· radical scavenging activity. From this fact, it assumed that the formation of intramolecular hydrogen bonding between carbonyl oxygen at acetoxyl group and 4-hydroxy group decreased the activity of glycosyl *p*-coumarates. Additionally, DPPH· radical scavenging activity of α-tocopherol decreased with the increasing concentration. It seemed that the solubility of α-tocopherol for the tested medium had a huge effect on the radical scavenging activity. The 0.1 μmol/mL solution of α-tocopherol showed clear and colorless. In contrast, the 0.5 and 1.0 μmol/mL solution was slightly suspended. This caused the low radical scavenging activity of 0.1 μmol/mL α-tocopherol solution compared with 0.5 and 1.0 μmol/mL.

3.4. Inhibitory effect on autoxidation of methyl linoleate

Figure 2 shows the inhibitory effect on autoxidation of bulk methyl linoleate using ferulic acids and glycosyl ferulate derivatives. Tested ferulic acid derivatives except *p*-coumaric acid and its glycosyl esters (**1b** and **2b**) showed higher antioxidant activity compared with α-tocopherol. The antioxidation activity decreased in the order sinapinic acid > glycosyl sinapinates (**1d** > **2d**) > ferulic acid ≈ glycosyl ferulates (**1c** ≈ **2c**) > *p*-coumaric acid ≈ glycosyl *p*-coumarates (**1b** ≈ **2b**). The antioxidant activity order against autoxidation of bulk methyl linoleate at 40°C was largely consistent with the scavenging activity order, suggesting that their antioxidant efficiencies result in their electron-donating ability. The results were in accordance with those reported by Pekkarinen *et al.* who measured the inhibition effects of some phenolic acids, including hydroxycinnamic acids, on the formation of hydroperoxides in bulk methyl linoleate at 40°C.[40] Cuvelier *et al.* evaluated the antioxidant activity of some phenolic acids based on measuring the disappearance of methyl linoleate in dodecane under heating (110°C). They reported that the activity order was caffeic acid > sinapinic acid > ferulic acid > *p*-coumaric acid, and the activity depended on their stability of aryloxy radicals.[43] Marinova and Yanishlieva also obtained the same activity order by determination of hydroperoxides, the primary oxidative products at 100°C.[44] From these results, there was no temperature dependent in the antioxidant activity of free ferulic acids against autoxidation of bulk methyl linoleate. The antioxidant activity between free ferulic acid and glycosyl ferulates showed a little difference. The esterification to glycosyl esters did not decrease the antioxidant activity of ferulic acid. The activity between free sinapinic acid and its glycosyl esters (**1d** and **2d**) was different. The antioxidant activity of free sinapinic acid and quercetin showed almost same. Free sinapinic acid exhibited higher antioxidant activity compared with glycosyl sinapinates (**1d** and **2d**). In glycosyl sinapinates (**1d** and **2d**), the antioxidant activity of **1d** was higher than that of **2d**. Kylli *et al.* evaluated the antioxidant activity of free sinapinic acid and its glycosyl ester and reported that the different.[39] It was reported that the affinities of antioxidants toward the air-oil interfaces in bulk oil affect antioxidant activity. These are in disagreement with our results. The activity strongly depended on the applied medium and reflected polar or apolar nature of the compound. It was stated, that polar antioxidant acts more effectively in apolar medium and apolar antioxidant in medium with polar solvent. **1d** possessed four acetoxyl group, and **2d** possessed two acetoxyl and two hydroxyl group. Therefore, the molecular polarity of glycosyl sinapinates (**1d** and **2d**) was different from free sinapinic acid. It seemed that this caused the difference of affinity toward the air-oil interfaces in bulk oil and affected the inhibitory effect on autoxidation of methyl linoleate. In the case of *p*-coumaric acid and its glycosyl esters, glycosyl *p*-coumarates (**1b** and **2b**) showed stronger activity than free *p*-coumaric acid at 40°C. Glycosyl *p*-coumarates (**1b** and **2b**) possessed hydroxyl group and/or acetoxyl group, and these groups increased molecular polarity. These results were in good agreement with the "polar paradox".

4. Conclusion

The synthesis of 2,3-di-*O*-acetyl-*β*-D-glucopyranosyl esters with carboxylic acids from 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide has been achieved via amine-promoted glycosylation procedure in which the *α*-D-glucose derivatives were converted to the corresponding glycosyl esters using amine without using the heavy metal compounds as catalysts. The resulting 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl esters were regioselectively deacetylated with Novozym 435. In the glycosyl esters **1a, 1b**, **1c** and **1d**, acetoxyl groups at C-4 and C-6 were regioselectively deacetylated. The regioselective deacetylation of only acetoxyl

groups at C-4 and C-6 in these compounds with Novozym 435 is primary interesting. Thus, the syntheses of 2,3-*O*-acetylglycosyl esters of the corresponding ferulic acids were accomplished without using the heavy metal catalysts, which are not only the cost-effective but also environmentally friendly method. The antioxidant properties of ferulic acids and its glycosyl esters were investigated. The antioxidant activities of ferulic acids and its glycosyl esters (**1c** and **2c**), as well as those of the other tested ferulic acids, were almost in agreement with their radical scavenging activities when measured using a bulk oil at 40°C. Sinapinic acid and its glycosyl esters (**1d** and **2d**) had the most effective antioxidant activity among tested ferulic acids against autoxidation of methyl linoleate. The esterification to glycosyl esters and subsequent regioselective deacelylation with lipase did not decrease the antioxidant activity of ferulic acid in oxidation of methyl linoleate and sinapinic acid in scavenging effect on $DPPH^{\cdot}$ radicals.

Acknowledgement

We thank Masaaki Kenmotsu, Mitsuru Kaminaga and Masashi Shoji for supporting this work. We are also grateful to the Novozymes A/S for the generous gift of Novozym 435. Our works were partly supported by the Academic Frontier Project for Private Universities: matching fund subsidy for MEXT (2007-2011), and the Research Project Grant B by Institute of Science and Technology, Meiji University, and Research Project Grant for Youth, Meiji University.

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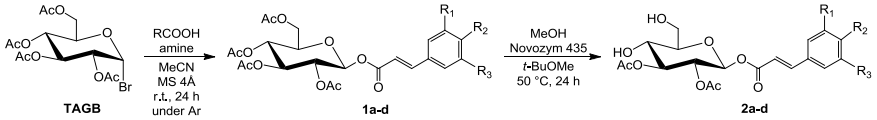
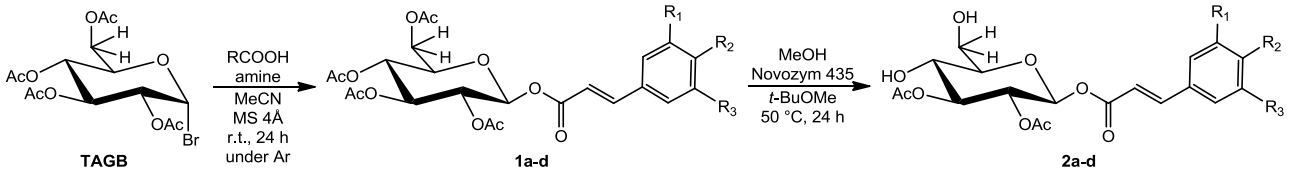
Graphic abstract

Synthesis of glycosyl ferulate derivatives by amine-promoted glycosylation with regioselective hydrolysis with Novozym 435 and evaluation of their antioxidant properties



a: $R_1 = R_2 = R_3 = H$ (*trans*-Cinnamic acid)
b: $R_1 = R_3 = H$, $R_2 = OH$ (*p*-Coumaric acid)
c: $R_1 = OMe$, $R_2 = OH$, $R_3 = H$ (Ferulic acid)
d: $R_1 = R_3 = OMe$, $R_2 = OH$ (Sinapinic acid)



a: $R_1 = R_2 = R_3 = H$ (*trans*-Cinnamic acid)
b: $R_1 = R_3 = H$, $R_2 = OH$ (*p*-Coumaric acid)
c: $R_1 = OMe$, $R_2 = OH$, $R_3 = H$ (Ferulic acid)
d: $R_1 = R_3 = OMe$, $R_2 = OH$ (Sinapi acid)

Scheme Synthesis of *O*-glycosyl ester

Table 1. Amine-promoted glycosylation of carboxylic acid with amine

Entry	Amine	Product	Yield [%]
1	*i*-Pr_2NEt	**1a**	72
2	*i*-Pr_2NEt	**1b**	55
3	Et_3N		81
4	*i*-Pr_2NEt	**1c**	74
5	*i*-Pr_2NEt	**1d**	73

Table 2. Novozym 435-catalyzed deacetylation

Entry	Solvent	Product	Yield [%]
1	*t*-BuOMe	**2a**	98
2	*t*-BuOMe[1)]	**2b**	65
3	*t*-BuOMe[1)]	**2c**	82
4	*t*-BuOMe	**2d**	90

1) *t*-BuOMe : PhH = 3 : 1

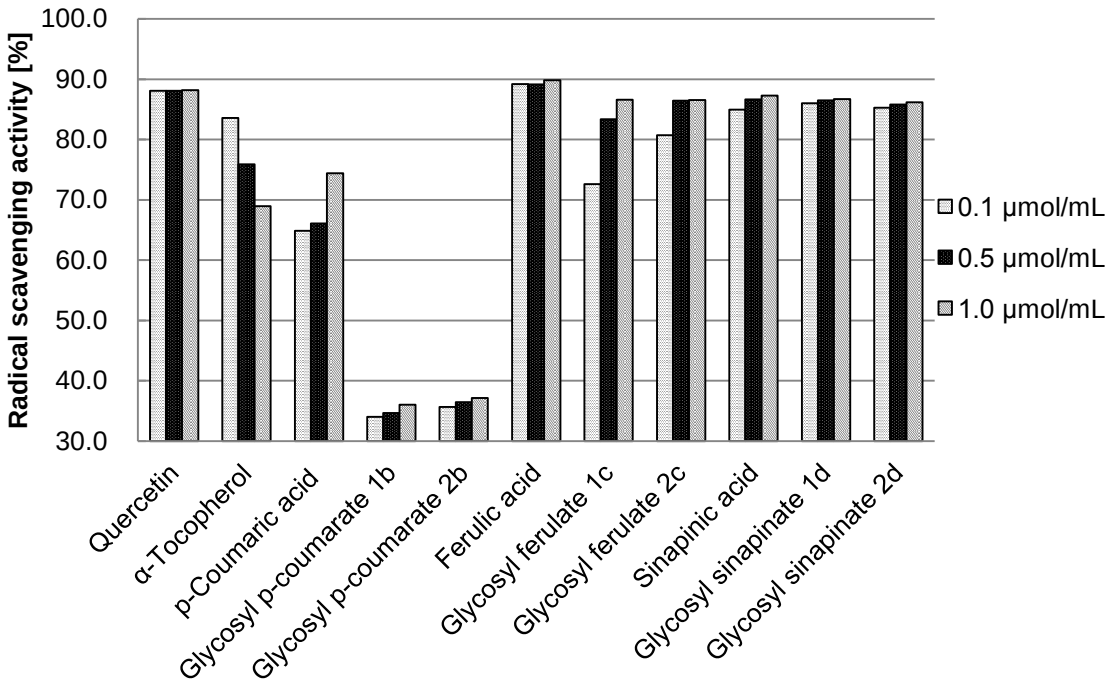


Figure 1 Radical scavenging activity (DPPH test) of free ferulic acids and its glycosyl esters

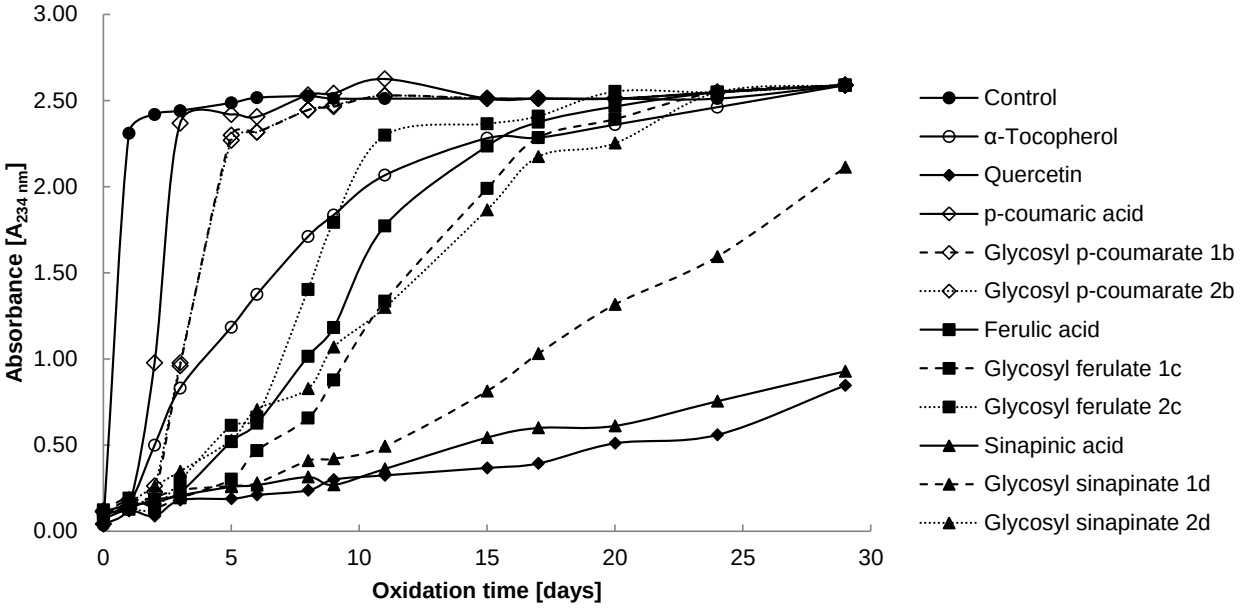


Figure 2 Inhibition of the formation of hydroperoxides in bulk methyl linoleate by ferulic acids and its glycosyl esters