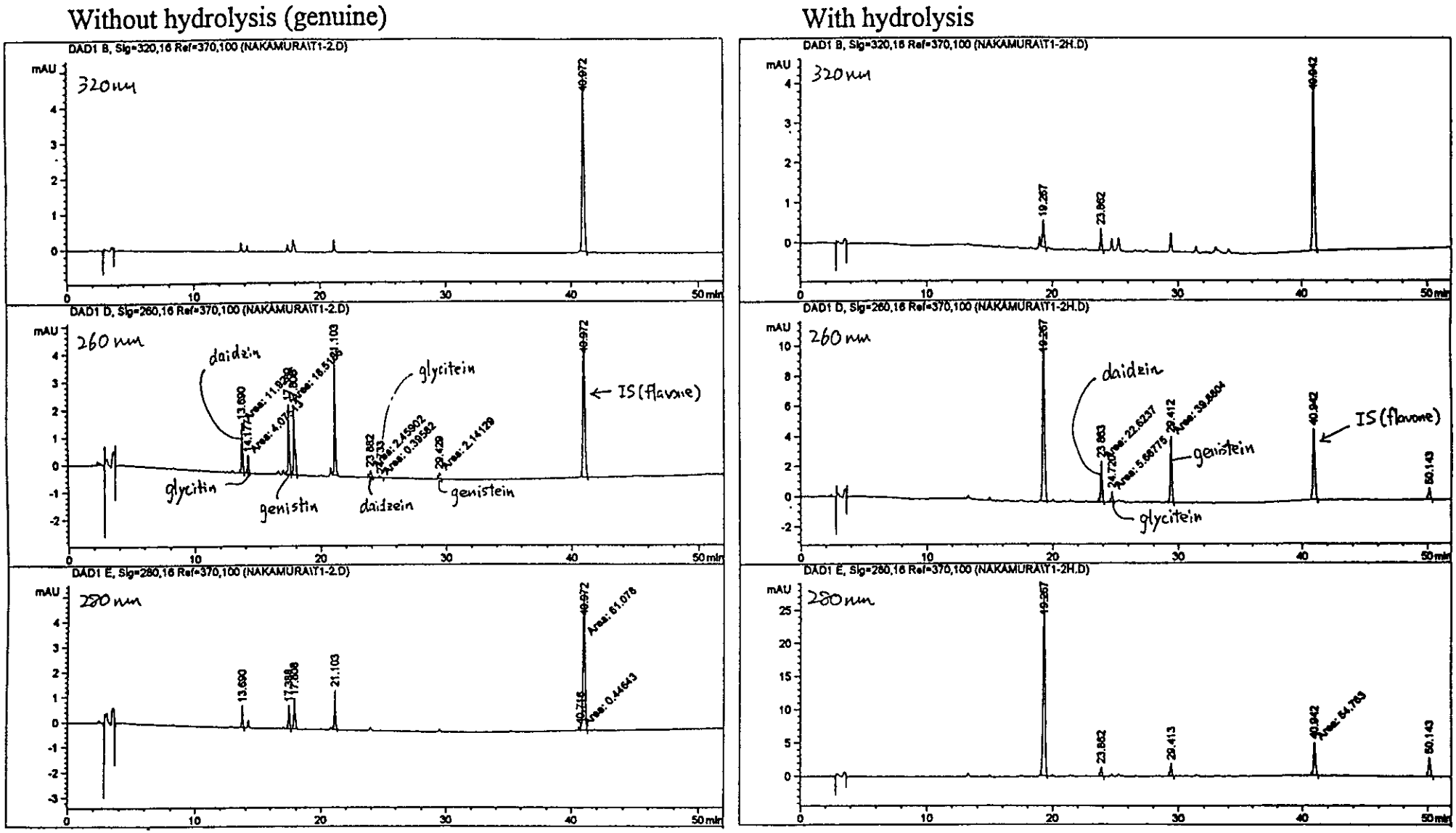


Figure 8 HPLC chromatograms of tofu

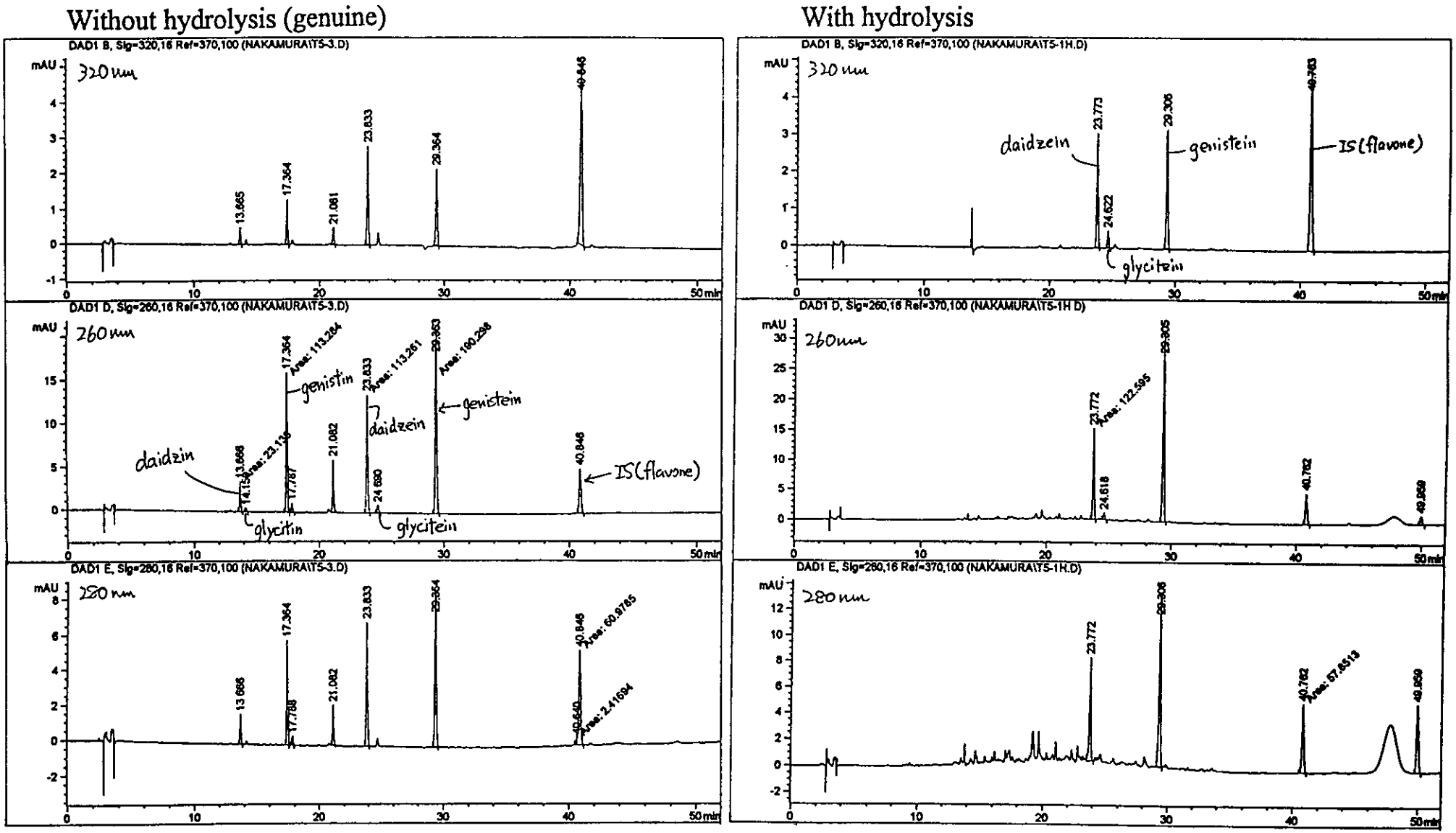


Figure 9 HPLC chromatograms of kori-dofu

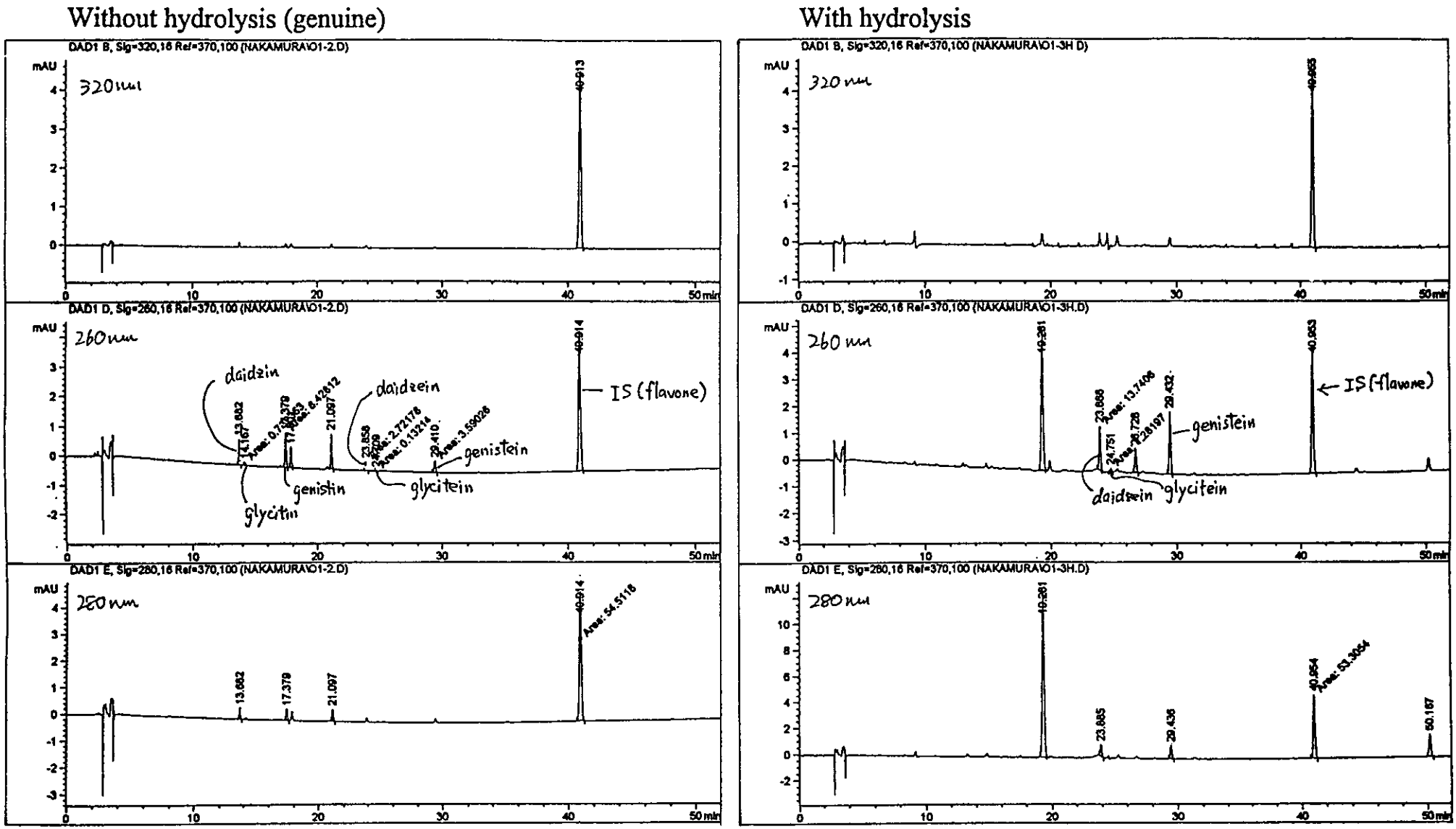


Figure 10 HPLC chromatograms of okara

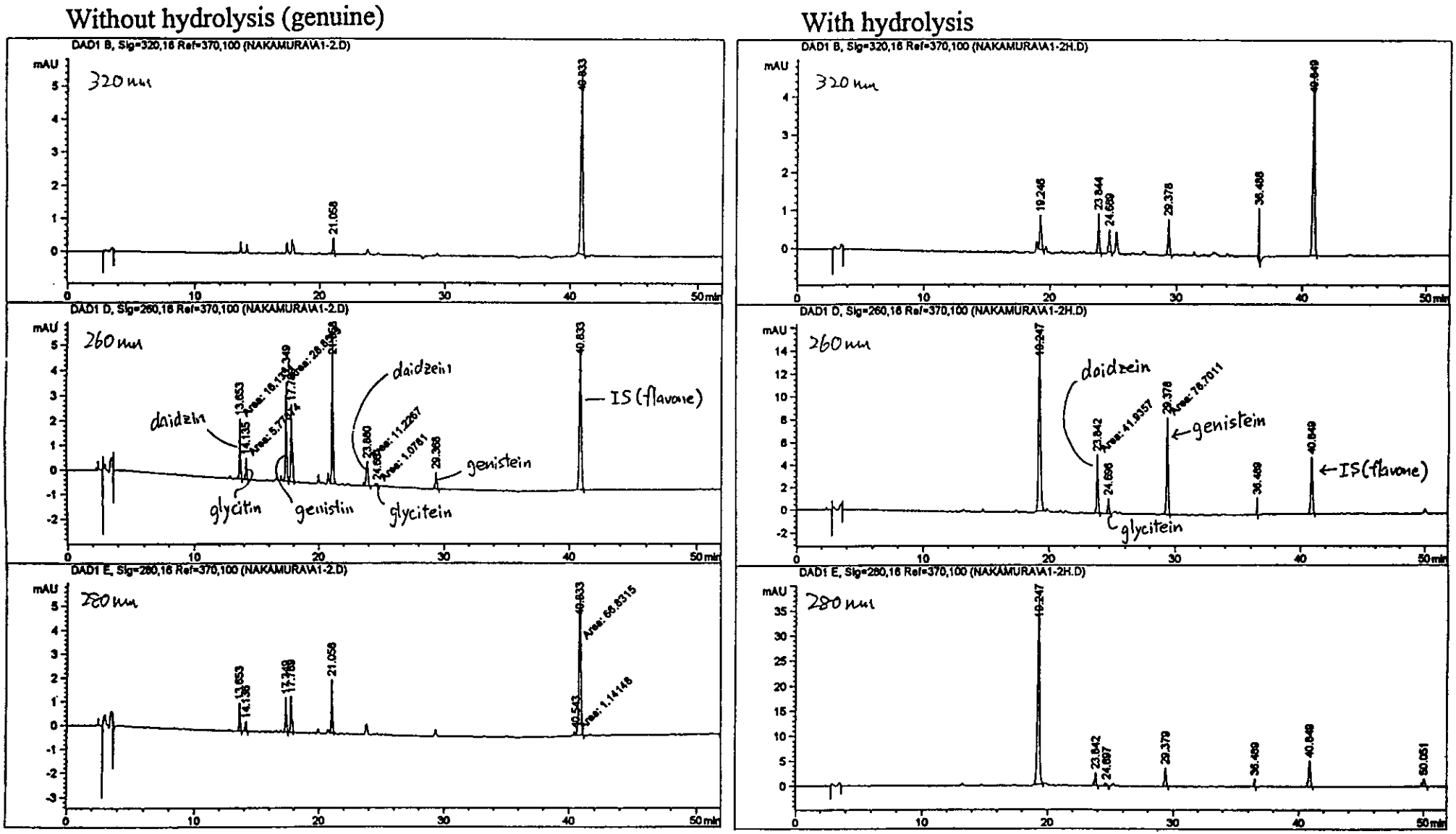


Figure 11 HPLC chromatograms of atsu-age

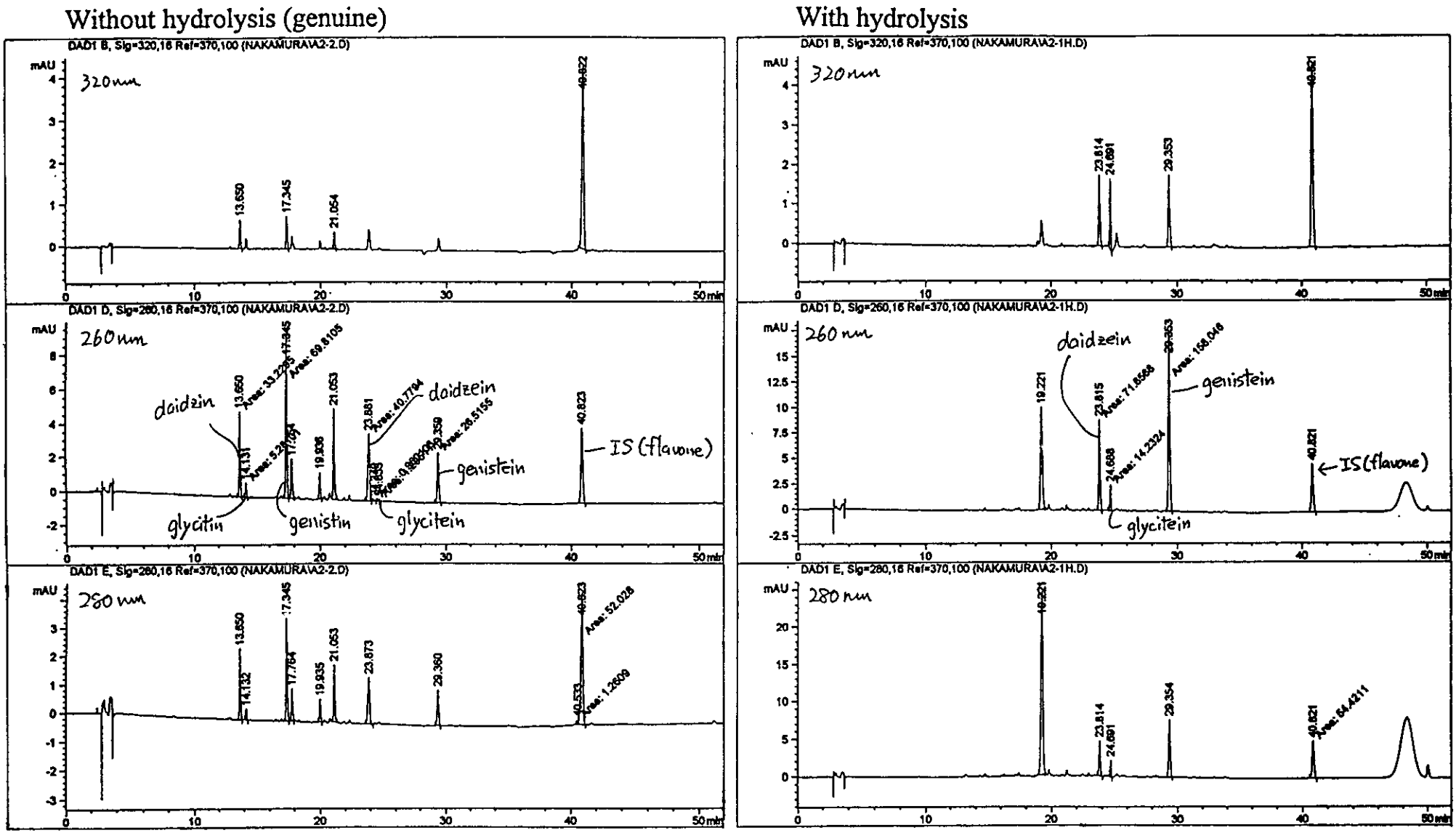


Figure 12 HPLC chromatograms of usu-age

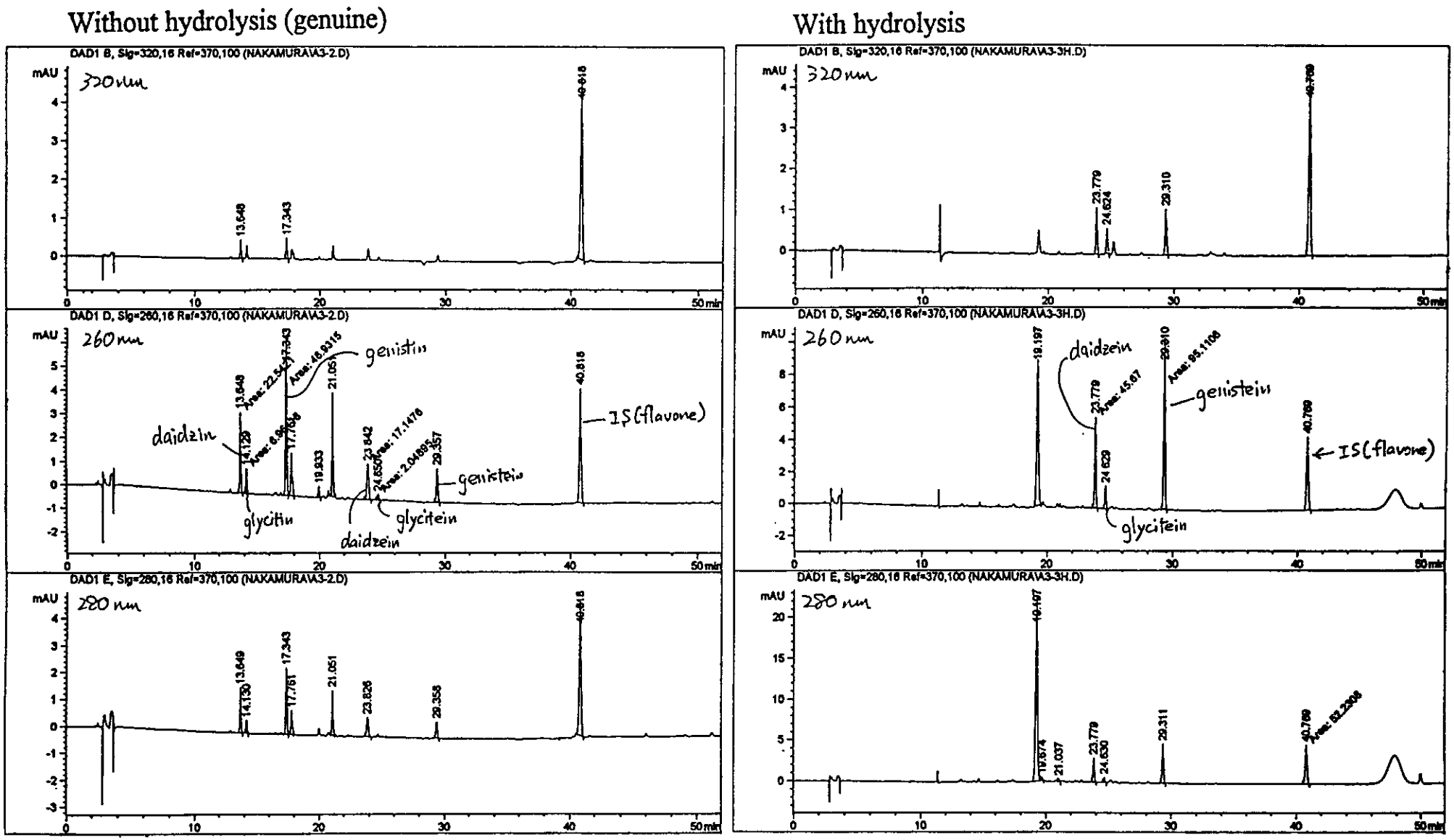


Figure 13 HPLC chromatograms of ganmodoki

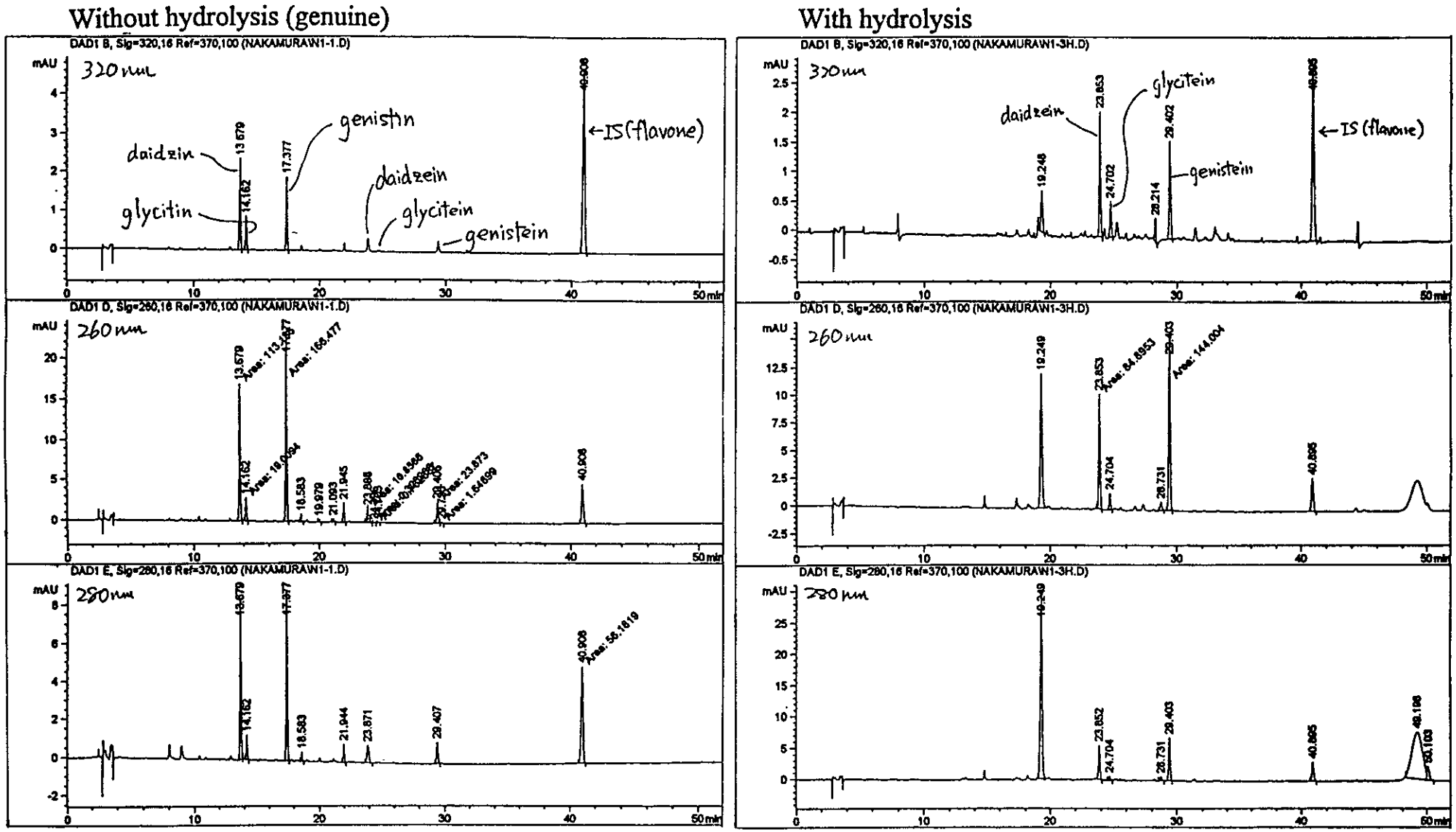


Figure 14 HPLC chromatograms of natto

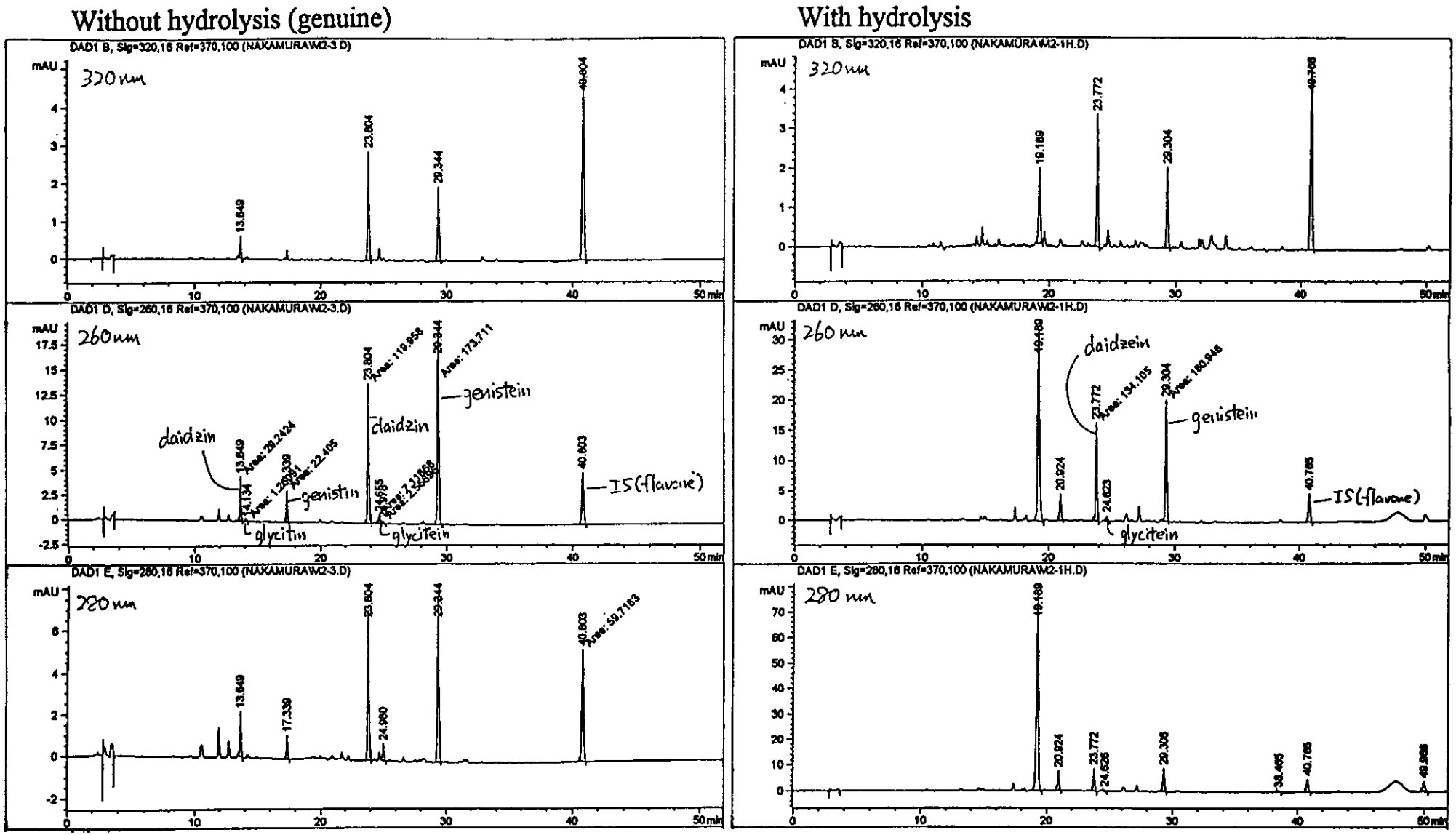


Figure 15 HPLC chromatograms of miso

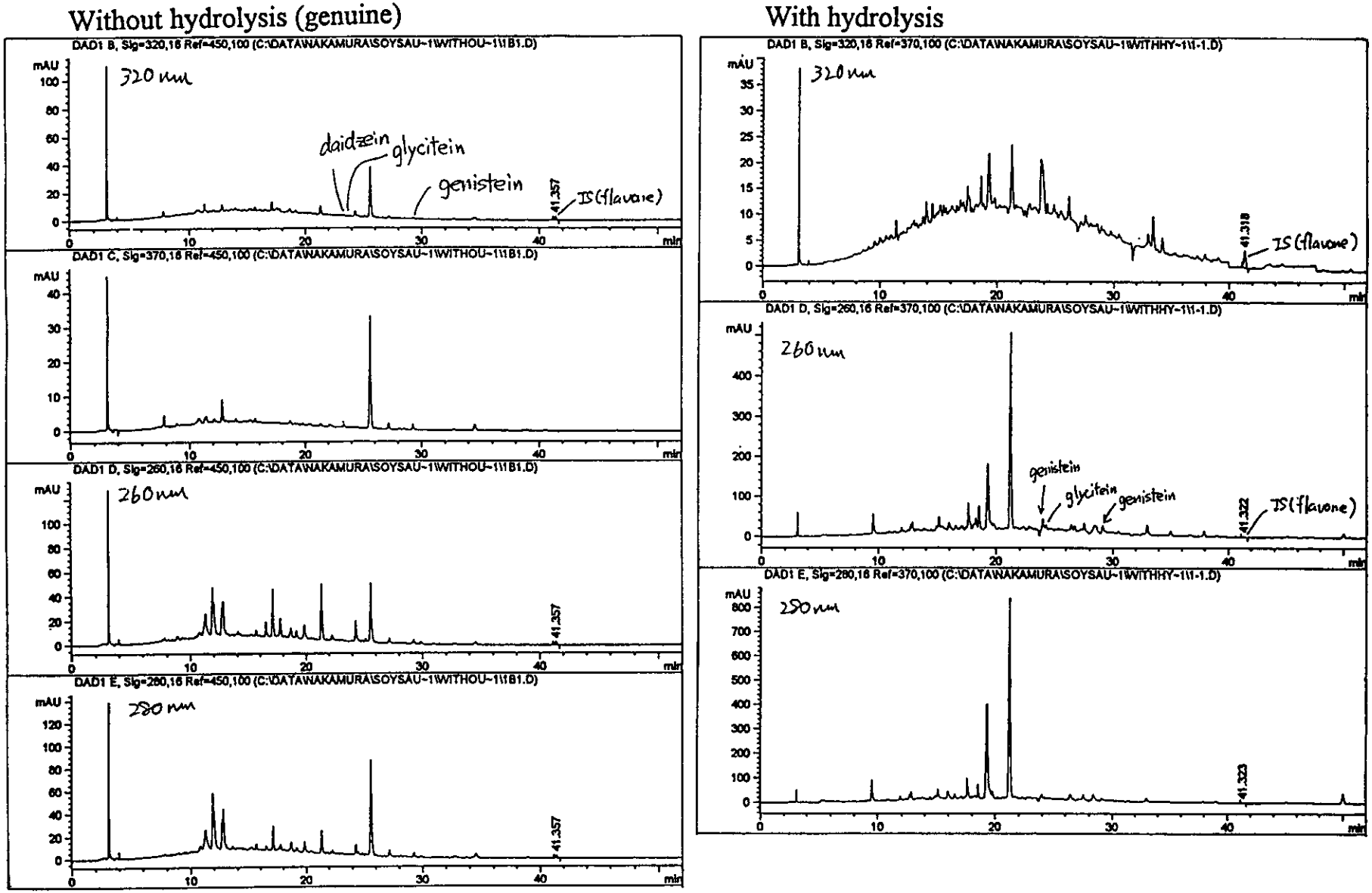


Figure 16 HPLC chromatograms of soy sauce

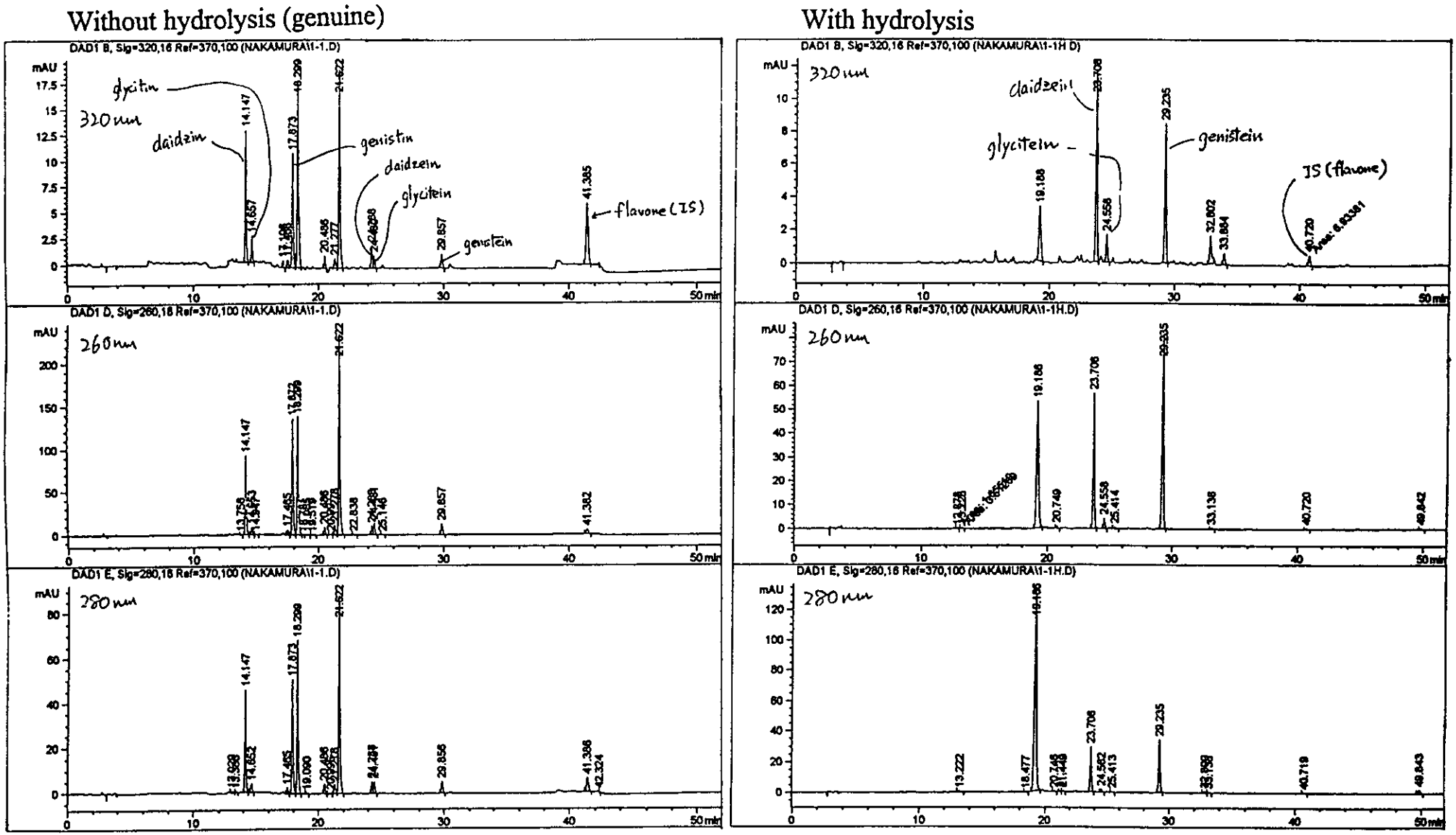


Figure 17 HPLC chromatograms of soy milk

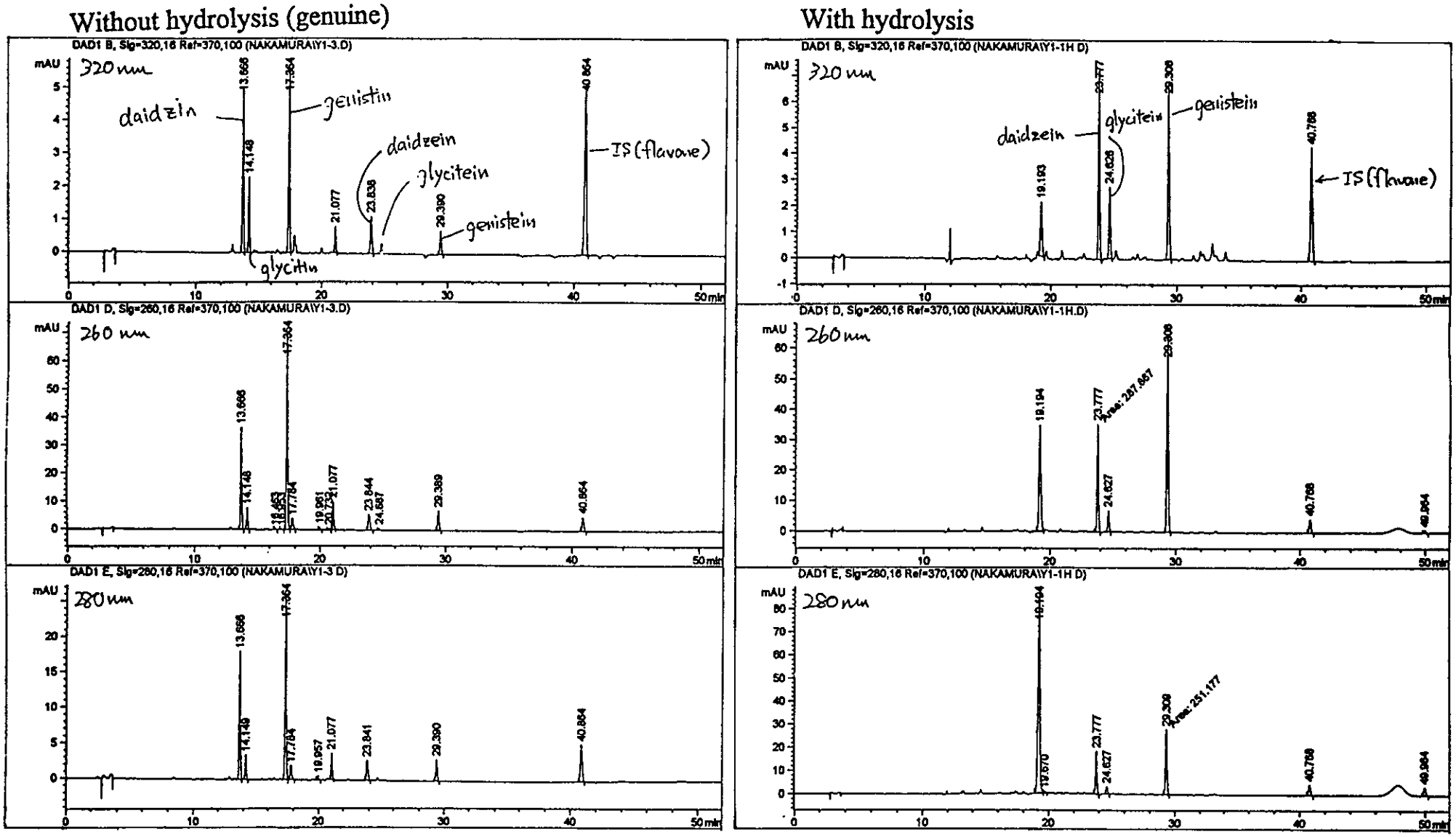


Figure 18 HPLC chromatograms of dried yuba

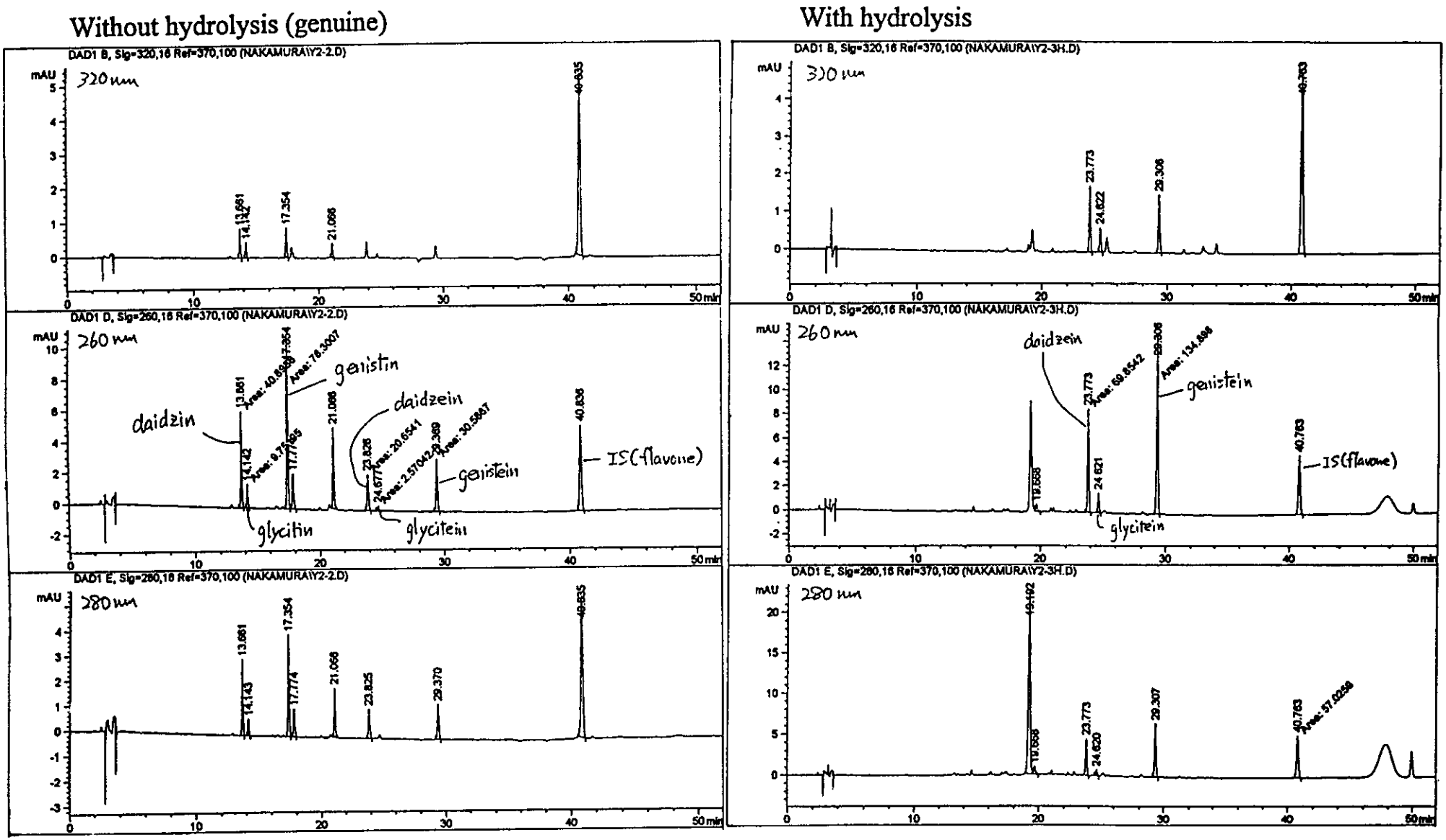


Figure 19 HPLC chromatograms of raw yuba

Table 1 Variation of several flavonoids by HPLC

| Flavonoids | Concentration [mmol/mL] | Retention time [min] | Peak area [mAU*s] |
|---|---|---|---|
| Daidzin | 21.73 | 13.80±0.06 | 353.93±3.24 |
| Glycitin | 29.55 | 14.29±0.06 | 346.91±11.43 |
| Genistin | 26.18 | 17.48±0.06 | 493.61±7.35 |
| Daidzein | 39.88 | 23.83±0.08 | 516.80±4.99 |
| Glycitein | 19.24 | 24.65±0.08 | 219.46±2.16 |
| Genistein | 40.70 | 29.34±0.09 | 757.06±13.36 |
| Equol | 28.32 | 29.63±0.09 | 72.91±0.99 |
| Formononetin | 38.54 | 33.86±0.09 | 502.35±2.60 |
| Biochanin A | 38.17 | 40.60±0.11 | 669.05±5.33 |
| Flavone | 47.16 | 40.83±0.09 | 394.93±6.08 |

Data are expressed as means±SD (n=10).
Conditions of HPLC are as follows.
Apparatus, HP 1100 series; column, STR ODSII (4.6 mmID×250 mm); column oven temperature, 35°C; Mobile phase, (solvent A) water: phosphoric acid 100:1 (v/v), (solvent B) water: acetonitrile: phosphoric acid 200:800:1 (v/v/v), (linear gradient program) B%: 10 (0 min) → 80 (50-52 min) → 10 (53 min); flow rate, 1.0 mL/min; detector, DAD; monitoring wavelength, 260 nm for daidzein, daidzin, genistein, genistin, glycitein, glycitin, biochanin A, formononetin, 280 nm for equol and flavone.

Table 2 Detection limit of several flavonoids by HPLC

| Flavonoids | Detection limit [pmol] |
|---|---|
| Daidzin | 0.1087 |
| Glycitin | 0.1478 |
| Genistin | 0.0524 |
| Daidzein | 0.0798 |
| Glycitein | 0.0385 |
| Genistein | 0.0814 |
| Equol | 0.5664 |
| Formononetin | 0.1929 |
| Biochanin A | 0.0763 |
| Flavone | 0.0943 |

Conditions of HPLC are as follows.
Apparatus, HP 1100 series; column, STR ODSII (4.6 mmID×250 mm); column oven temperature, 35°C; Mobile phase, (solvent A) water: phosphoric acid 100:1 (v/v), (solvent B) water: acetonitrile: phosphoric acid 200:800:1 (v/v/v), (linear gradient program) B%: 10 (0 min) → 80 (50-52 min) → 10 (53 min); flow rate, 1.0 mL/min; detector, DAD; monitoring wavelength, 260 nm for daidzein, daidzin, genistein, genistin, glycitein, glycitin, biochanin A, formononetin, 280 nm for equol and flavone.

Table 3 Recoveries of standard solutions of flavonoids from Sep-pak plus $C_{18}{}^{R}$ cartridge

| Flavonoids | Spiked amounts [nmol] | Recovery [%] Standard |
|---|---|---|
| Daidzin | 8.69 | 102.44±1.21 |
| Glycitin | 11.82 | 106.27±1.49 |
| Genistin | 10.47 | 102.23±1.85 |
| Daidzein | 15.95 | 102.66±2.08 |
| Glycitein | 7.70 | 99.56±1.84 |
| Genistein | 16.28 | 104.20±0.07 |
| Equol | 11.33 | 102.85±1.72 |
| Formononetin | 15.42 | 102.85±1.72 |
| Biochanin A | 15.27 | 103.84±0.98 |

Data are represented as means±SD (n=3).
Each flavonoid was added onto a Sep-pak plus $C_{18}{}^{R}$ cartridge column preconditioned by 10 mL of methanol followed by 10 mL of distilled water. The column was washed with 10 mL of water followed by 2 mL of 20% methanol and flavonoid was eluted by 2 mL of methanol. Eluate was evaporated and redissolved with 2 mL of water. Each flaonoid was determined by HPLC.

Table 4 Recoveries of flavonoids with or without hydrolysis from soy sauce

| Flavonoids | Spiked amounts [nmol/mL] | Recovery [%] Without hydrolysis | With hydrolysis |
|---|---|---|---|
| Daidzin | 8.69 | 90.57±6.94 | 100.52±8.78 |
| Glycitin | 11.82 | 94.19±6.07 | 90.82±7.37 |
| Genistin | 10.47 | 108.18±3.71 | 92.28±9.39 |
| Daidzein | 15.95 | 81.16±7.95 | 80.86±7.58 |
| Glycitein | 7.70 | 105.23±4.07 | 66.20±6.08 |
| Genistein | 16.28 | 108.63±2.51 | 82.67±7.85 |
| Equol | 11.33 | 102.73±4.99 | 101.93±3.02 |
| Formononetin | 15.42 | 103.52±1.13 | 80.13±7.06 |
| Biochanin A | 15.27 | 101.33±4.43 | 68.83±5.56 |

Data are represented as means±SD (n=3).
(1) Without hydrolysis: To one mL of soy sauce, flavone 94.3 nmol as an internal standard and/or each flavonoid was added. They were added onto a Sep-pak plus $C_{18}{}^{R}$ cartridge column preconditioned with 10 mL of methanol followed by 10 mL of distilled water. The column was washed with 10 mL of water followed by 2 mL of 20% methanol, and the flavonoid was eluted with exactly 2 mL of methanol. Each flaonoid was determined by HPLC.
(2) With hydrolysis: To five mL of soy sauce, flavone 94.3 nmol as an internal standard and/or each flavonoid, ten mL of 10N HCl and forty mL of 95.5% ethanol containing 0.05% BHT as an antioxidant were added. Hydrolysis was performed at 100°C for 3 h. Hydrolysate was cooled to a room temperature and centrifuged at 1,000 *g* for 15 min. Supernatant was adjusted to 50 mL with ethanol, then 10 mL was evaporated under the nitrogen stream and redissolved with 10 mL of water and added onto a Sep-pak plus $C_{18}{}^{R}$ cartridge column preconditioned with methanol and water. The column was washed with 10 mL of water followed by 2 mL of 20% methanol, and the flavonoid was eluted with exactly 2 mL of methanol. Each flaonoid was determined by HPLC.

Table 5 Recoveries of flavonoids with or without hydrolysis from soy milk

| Flavonoids | Spiked amounts [nmol/mL] | Recovery [%] Without hydrolysis | With hydrolysis |
|---|---|---|---|
| Daidzin | 17.38 | 90.63±9.08 | 109.87 |
| Glycitin | 23.64 | 83.79±4.60 | 89.31±1.00 |
| Genistin | 20.94 | 87.51±2.23 | 84.24 |
| Daidzein | 31.90 | 85.54±7.83 | 65.03 |
| Glycitein | 15.40 | 92.88±3.97 | 66.70±3.17 |
| Genistein | 32.56 | 83.03±2.63 | 91.93 |
| Equol | 22.66 | 88.59±4.28 | 103.03±8.55 |
| Formononetin | 30.84 | 86.28±3.37 | 106.97±2.35 |
| Biochanin A | 30.54 | 79.69±4.10 | 94.81 |

Data are represented as means±SD (n=3).
(1) Without hydrolysis: To 0.5 mL of soy milk, flavone 94.3 nmol as an internal standard and/or each flavonoid was added. They were added onto a Sep-pak plus $C_{18}{}^{R}$ cartridge column preconditioned with 10 mL of methanol followed by 10 mL of distilled water. The column was washed with 10 mL of water followed by 2 mL of 20% methanol, and the flavonoid was eluted with exactly 2 mL of methanol. Each flaonoid was determined by HPLC.
(2) With hydrolysis: To five mL of soy milk, flavone 94.3 nmol as an internal standard and/or each flavonoid, ten mL of 10N HCl and forty mL of 95.5% ethanol containing 0.05% BHT as an antioxidant were added. Hydrolysis was performed at 100°C for 3 h. Hydrolysate was cooled to a room temperature and centrifuged at 1,000 *g* for 15 min. Supernatant was adjusted to 50 mL with ethanol, then 10 mL was evaporated under the nitrogen stream and redissolved with 10 mL of water and added onto a Sep-pak plus $C_{18}{}^{R}$ cartridge column preconditioned with methanol and water. The column was washed with 10 mL of water followed by 2 mL of 20% methanol, and the flavonoid was eluted with exactly 2 mL of methanol. Each flaonoid was determined by HPLC.

Table 6 Recoveries of flavonoids with or without hydrolysis from soy bean

| Flavonoids | Spiked amounts [nmol/g] | Recovery [%] Without hydrolysis | With hydrolysis |
|---|---|---|---|
| Daidzin | 434.6 | 97.25±4.33 | 94.97±9.19 |
| Glycitin | 295.1 | 94.29±7.96 | 90.35±4.31 |
| Genistin | 523.6 | 97.28±6.56 | 96.05±9.04 |
| Daidzein | 797.6 | 106.78±9.75 | 98.28±8.18 |
| Glycitein | 192.4 | 94.55±1.64 | 90.47±6.44 |
| Genistein | 814.1 | 112.60±5.98 | 103.62±5.96 |
| Equol | 283.2 | 103.52±2.44 | 74.68±0.63 |
| Formononetin | 385.4 | 102.38±2.50 | 110.47±7.52 |
| Biochanin A | 381.7 | 105.73±4.22 | 114.14±6.21 |

Data are represented as means±SD (n=3).
(1) Without hydrolysis: To one g of ground soy bean powder, flavone 943 nmol as an internal standard and/or each flavonoid was added. Flavonoids were extracted with 50 mL of 80% methanol for 24 h after sonification, then centrifuged at 800 *g* for 15 min. The supernatant was adjusted to 50 mL with methanol (extract 1). One mL of the extract 1 was diluted to 10 mL with water and added onto a Sep-pak plus $C_{18}{}^{R}$ cartridge column preconditioned with 10 mL of methanol followed by 10 mL of distilled water. The column was washed with 10 mL of water followed by 2 mL of 20% methanol, and the flavonoid was eluted with exactly 2 mL of methanol. Each flaonoid was determined by HPLC.
(2) With hydrolysis: To one g of ground soy bean powder, flavone 943 nmol as an internal standard and/or each flavonoid, ten mL of 10N HCl and forty mL of 95.5% ethanol containing 0.05% BHT as an antioxidant were added. Hydrolysis was performed at 100°C for 3 h. Hydrolysate was cooled to a room temperature and centrifuged at 1,000 *g* for 15 min. Supernatant was adjusted to 50 mL with ethanol (extract 2). One mL of the extract 2 was diluted to 10 mL with water and added onto a Sep-pak plus $C_{18}{}^{R}$ cartridge column preconditioned with methanol and water. The column was washed with 10 mL of water followed by 2 mL of 20% methanol, and the flavonoid was eluted with exactly 2 mL of methanol. Each flaonoid was determined by HPLC.

Table 7 Recoveries of flavonoids with or without hydrolysis from tofu

| Flavonoids | Spiked amounts [nmol/g] | Recovery [%] Without hydrolysis | With hydrolysis |
|---|---|---|---|
| Daidzin | 434.6 | 102.82±0.57 | |
| Glycitin | 295.1 | 102.12±0.92 | |
| Genistin | 523.6 | 100.61±0.28 | |
| Daidzein | 797.6 | 105.77±1.71 | |
| Glycitein | 192.4 | 106.54±3.04 | |
| Genistein | 814.1 | 102.65±1.24 | |
| Equol | 283.2 | 111.01±1.79 | |
| Formononetin | 385.4 | 106.85±1.76 | |
| Biochanin A | 381.7 | 94.09±3.44 | |

Data are represented as means±SD (n=3).
(1) Without hydrolysis: To one g of crushed tofu, flavone 943 nmol as an internal standard and/or each flavonoid was added. Flavonoids were extracted with 50 mL of 80% methanol for 24 h after sonification, then centrifuged at 800 *g* for 15 min. The supernatant was adjusted to 50 mL with methanol (extract 1). One mL of the extract 1 was diluted to 10 mL with water and added onto a Sep-pak plus $C_{18}{}^{R}$ cartridge column preconditioned with 10 mL of methanol followed by 10 mL of distilled water. The column was washed with 10 mL of water followed by 2 mL of 20% methanol, and the flavonoid was eluted with exactly 2 mL of methanol. Each flaonoid was determined by HPLC.

Table 8 Recoveries of flavonoids with or without hydrolysis from miso

| Flavonoids | Spiked amounts [nmol/g] | Recovery [%] Without hydrolysis | With hydrolysis |
|---|---|---|---|
| Daidzin | 434.6 | 100.95±5.94 | 99.04±9.11 |
| Glycitin | 295.1 | 110.35±1.74 | 96.85±2.80 |
| Genistin | 523.6 | 105.21±0.11 | 93.67±6.25 |
| Daidzein | 797.6 | | 94.94±0.03 |
| Glycitein | 192.4 | | 100.57±0.27 |
| Genistein | 814.1 | | 81.90±1.68 |
| Equol | 283.2 | | 63.46±2.17 |
| Formononetin | 385.4 | | 104.05±1.63 |
| Biochanin A | 381.7 | | 91.64±9.17 |

Data are represented as means±SD (n=3).
(1) Without hydrolysis: To one g of ground miso, flavone 943 nmol as an internal standard and/or each flavonoid was added. Flavonoids were extracted with 50 mL of 80% methanol for 24 h after sonification, then centrifuged at 800 *g* for 15 min. The supernatant was adjusted to 50 mL with methanol (extract 1). One mL of the extract 1 was diluted to 10 mL with water and added onto a Sep-pak plus $C_{18}{}^{R}$ cartridge column preconditioned with 10 mL of methanol followed by 10 mL of distilled water. The column was washed with 10 mL of water followed by 2 mL of 20% methanol, and the flavonoid was eluted with exactly 2 mL of methanol. Each flaonoid was determined by HPLC.
(2) With hydrolysis: To one g of ground miso, flavone 943 nmol as an internal standard and/or each flavonoid, ten mL of 10N HCl and forty mL of 95.5% ethanol containing 0.05% BHT as an antioxidant were added. Hydrolysis was performed at 100°C for 3 h. Hydrolysate was cooled to a room temperature and centrifuged at 1,000 *g* for 15 min. Supernatant was adjusted to 50 mL with ethanol (extract 2). One mL of the extract 2 was diluted to 10 mL with water and added onto a Sep-pak plus $C_{18}{}^{R}$ cartridge column preconditioned with methanol and water. The column was washed with 10 mL of water followed by 2 mL of 20% methanol, and the flavonoid was eluted with exactly 2 mL of methanol. Each flaonoid was determined by HPLC.