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Identification and Characterization of Phytochemicals from Peanut (*Arachis hypogaea* L.) Pods

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Abstract Methanol extracts of peanut (*Arachis hypogaea* L.) pods were chromatographed, which yielded 3 phytochemicals **1-3** including 5,7-dihydroxychromone (**1**), eriodictyol (**2**), and 3',4',5,7-tetrahydroxyflavone (**3**). To confirm the presence of isolated phytochemicals, the pods extracts were performed by high performance liquid chromatography coupled with a photodiode array detector (HPLC-PDA) and a mass spectrometric detector (MSD) with electrospray ionization (ESI). Optimum extraction conditions for phytochemical contents using peanut germplasm were obtained by employing 90% MeOH for 12 hr at room temperature and phytochemicals **1-3** showed significant differences with concentrations of 407.56±23.35, 52.92±5.11, and 2,024.34±134.18 μg/g, respectively. Under this optimal conditions, the contents of phytochemicals **1-3** in peanut pods of 3 Korea cultivars including 'Jakwang', 'Daekwang', and 'Palkwang' exhibited phytochemical **3** was the highest range of 1,338.01-5,162.93 μg/g, followed by phytochemical **1** (590.13-1,382.10 μg/g), and phytochemical **2** (25.12-186.85 μg/g), respectively. Moreover, 'Jakwang' exhibited the highest contents of phytochemical (**1**: 1,362.10±52.49, **2**: 186.85±17.69, and **3**: 5,162.93±148.64 μg/g, respectively), whereas the lowest contents was found in the 'Daekwang' (**1**: 590.13±22.23, **2**: 25.12±2.45, and **3**: 1,338.01±62.17 μg/g, respectively). These results suggest that the methanol extracts of peanut pods may possess health related benefits to humans owing to various known biological activities of phytochemicals **1-3**.

Keywords: peanut pod, phytochemical, 5,7-dihydroxychromone, eriodictyol, 3',4',5,7-tetrahydroxyflavone, high performance liquid chromatography (HPLC), extraction condition

Introduction

Extraction and characterization of phytochemicals with antioxidative, cancer chemopreventive activities have attracted extensive interest from individuals involved in biomedical research and development (1-5). The identification, quantification, and characterization of phytochemicals are important for applications of plant extracts as new food additives, developments of efficient quality control measures to ensure the authenticity and standardization of product composition and quality.

Peanut (*Arachis hypogaea* L.) represent one of the major vegetable crops grown, worldwide, constituting an important part of a well balanced diet and numerous potential dietary benefits (6-9). Recently, peanut cultivars were developed with elevated concentrations of the oleic acid with greater health benefits and serves to prolong shelf life characteristics (10,11). In addition, many phytochemicals of this species have had unprecedented attention due to potential antioxidant capacities (6,7,12) such as stilbene, flavanone, and proanthocyanidin, as well as tocopherol and protein (13,14). It is well established that seeds, skins, kernels, roots, and hulls of peanut contain abundant secondary metabolites possessing antioxidant activity (15-18). Although there are phytochemicals present in pods of peanut (19,20), researchers have not been widely attempted. Also, extraction conditions and characterization of phytochemicals from this species have not been studied. Among phytochemicals, 5,7-dihydroxychromone, eriodictyol, and 3',4',5,7-tetrahydroxyflavone (luteolin) are particularly interesting flavonoids owing to their wide distribution in common foods, various fruits, and vegetables. 5,7-Dihydroxychromone treats intestinal infections (21) and eriodictyol has been seen to significantly inhibit lipid peroxidation (22) as well as active against several tumor cell lines (23,24). 3',4',5,7-Tetrahydroxyflavone exhibits a wide spectrum of pharmacological properties including anti-inflammatory and anti-allergic properties (25) as well as possess effect such as vasodilatation (26) and antioxidation (27). Thus, the evaluation of phytochemicals in the seeds and different parts of peanut are great importance not only the value of the whole plant as a source of bioactive materials but also that of peanut as dietary supplement.

In the present study, to obtain information on the

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phytochemical compositions from the pods of peanut, 3 phytochemicals including 5,7-dihydroxychromone (**1**), eriodictyol (**2**), and 3',4',5,7-tetrahydroxyflavone were isolated (**3**). Furthermore, the isolated phytochemical contents and the optimal extraction conditions by high performance liquid chromatography (HPLC) diode array detection and liquid chromatography/mass spectrometry (LC/MS) with positive and negative electrospray ionization were investigated. The variation of these component contents from Korea cultivars was also reported.

Materials and Methods

Plant material For analysis of optimal extraction conditions from peanut pods, peanut was sampled in Korea germplasms. Three species used in this experiment were *A. hypogaea* cv. Daekwang, Palkwang, and Jakwang. All these samples were collected on September 12, 2006, in the experimental field of the Yeongnam Agricultural Research Institute (YARI), National Institute of Crop Science, Rural Development Administration (RDA), Miryang, Korea. The harvested samples were stored at −70°C until analysis.

Reagents HPLC-grade water (99.9%, v/v) and acetonitrile (99.9%, v/v) were from Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA, 99.8%, v/v) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents used throughout the experiments were of analytical grade.

Instruments The purity of all phytochemicals were monitored by thin layer chromatography (TLC) (Merck Co., Darmstadt, Germany), using commercially available glass-backed plates and visualized under ultra violet (UV) at 254 and 366 nm or sprayed with a 10% ethanolic solution of phosphomolybdic acid (PMA) (Wako Pure Chemical Industries, Osaka, Japan). Column chromatography was carried out using 230-400 mesh silica gel (Kieselgel 60, Merck). Melting points were measured on a Thomas Scientific capillary melting point apparatus (Electrothermal 9300; Manchester, UK) and are uncorrected. Infrared (IR) spectra were recorded on a Bruker IFS66 (Bruker, Karlsruhe, Germany) infrared Fourier transform spectrophotometer (KBr) and UV spectra were measured on a Beckman DU650 spectrophotometer (Beckman Coulter, Fullerton, CA, USA). ^{1}H- and ^{13}C-NMR along with 2D-NMR data were obtained on a Bruker AM 500 (^{1}H-NMR at 500 MHz, ^{13}C-NMR at 125 MHz) spectrometer (Bruker) in DMSO-d_6. Electron impact mass spectroscopy (EIMS) was obtained on a Jeol JMS-700 mass spectrometer (Jeol, Tokyo, Japan). Chromatographic separation was achieved using an Agilent 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary HPLC pump, a degasser, an autosampler and a single wavelength UV detector. Analysis of isolated phytochemicals was carried out using reverse phase separation on a C_{18} column (LichroCART 125-4 HPLC-Cartridge, Lichrophore 100 RP-18e; Merck). The system was coupled to DE/ESQUIRE 4000 LC/MS (Bruker)

Extraction and isolation The dried pods of peanut (2.1 kg, peanut germplasm in Korea) were pulverized (600 mesh) using a grinder and extracted with methanol (7 L) for 10 days at room temperature. The combined methanol was concentrated *in vacuo* to yield a brown gum (26 g). The methanol extracts was dissolved in 300 mL of a mixture of water and successively partitioned with *n*-hexane, EtOAc, and *n*-BuOH yielding *n*-hexane (1.7 g), EtOAc (4.9 g), *n*-BuOH (6.2 g), and H_2O extracts (8.1 g), respectively. The EtOAc phase was chromatographed on a silica gel column (3.0×40 cm, 230-400 mesh, 210 g) using *n*-hexane/acetone [25:1 (300 mL), 20:1 (300 mL), 16:1 (200 mL), 12:1 (200 mL), 8:1 (200 mL), 4:1 (200 mL)] mixtures to give fractions A (900 mg), B (650 mg), C (540 mg), D (470 mg), and E (510 mg), respectively. Fraction C was applied to a silica gel column (2.5×40 cm, 230-400 mesh, 140 g) chromatography with *n*-hexane/acetone (15:1 → 4:1) and then purified by a second flash silica gel column (1.5×30 cm, 230-400 mesh, 40 g) using a gradient of *n*-hexane/acetone [12:1 (80 mL), 8:1 (80 mL), 5:1 (80 mL), 3:1 (80 mL)] to yield phytochemical **1** (35 mg). The BuOH phase was chromatographed on silica gel (3.5×50 cm, 230-400 mesh, 270 g) using a gradient of $CHCl_3$/acetone to give fraction A-F. Fraction C (390 mg) was applied to a silica gel column chromatograph with $CHCl_3$/acetone [20:1 (300 mL), 15:1 (200 mL), 12:1 (100 mL), 10:1 (100 mL), 8:1 (100 mL), 6:1 (100 mL), 3:1 (100 mL), 1:1 (100 mL)] as a mobile phase to afford 39 subfractions. Subfractions 28-34 were pooled and rechromatographed on silical gel with a $CHCl_3$/acetone to give phytochemical **2** (47 mg). Fraction D (340 mg) was chromatographed using a stepwise gradient of $CHCl_3$/acetone [15:1 (250 mL), 12:1 (150 mL), 10:1 (150 mL), 8:1 (150 mL), 6:1 (150 mL), 3:1 (150 mL), 1:1 (150 mL)], then purified by second flash silica gel column (2.0×30 cm, 230-400 mesh, 90 g) using a gradient of $CHCl_3$/acetone [10:1 (80 mL), 8:1 (80 mL), 6:1 (80 mL), 4:1 (80 mL), 2:1 (80 mL), 1:1 (80 mL)] to yield phytochemical **3** (41 mg).

Sample preparation, HPLC, and LC/MS analysis conditions For analysis of the 3 phytochemicals, 1.0 g of freeze-dried finely ground pods of peanut was put into a test tube, into which 10 mL of 90% MeOH was added. The solution was then mixed 12 hr with a vortex mixer at room temperature. The extracts were filtered through Whatman No. 42 filter paper and then the extracts used for HPLC analysis were passed through a 0.45-mm filter (MSI; Millipore, Westboro, MA, USA). HPLC separation and quantification of isolated phytochemicals were performed on a Agilent 1100 series instrument equipped with photodiode array detector (PDA) using a reverse-phase column. The mobile phase for HPLC consisted of solvent A, 0.1% TFA in water, and solvent B, 100% acetonitrile. The solvent gradient was as follows: 0 min, 10% B; 20 min, 20% B; 30 min, 25% B; 35 min, 35% B and then held for 10 min before returning to the initial conditions. The flow rate was 1.0 mL/min and the injection was volume 20 mL. The eluted extract was detected at 254 nm and all HPLC analyses were performed at 30°C. Conditions of LC/MS detection were as follows: target mass (positive and negative ionization modes), 100%; drying gas (N_2 from generator) flow rate, 1.0 mL/min; gas temperature, 350°C; pressure, 30 psi; and collision gas He pressure, 6×10^{-6} mbar. Data was acquired in the electronspray impact (ESI) mode with a scan range of 100-320.

Calibration curves of 3 phytochemicals Approximately 2 mg of purified phytochemicals were accurately weighted and dissolved in a 10 mL volumetric flask in 90% MeOH to obtain stock solution. For calibration curves, the stock solution was diluted with 90% MeOH to obtain at 8 concentrations sequence (125, 100, 50, 25, 12, 6, 3, and 1 μg/mL), and a high linearity of R^2>0.997 was obtained from each curve. Three purified phytochemicals were identified by their retention times and concentrations were calculated by comparing the peak areas of samples with those of the standards (mean areas, n=3).

Statistical analysis The peanut was cultivated using a completely randomized design and the analysis of the phytochemicals by HPLC was repeated 3 in each cultivar. The analyses of data were performed using Sigma Plot 2001.

Results and Discussion

Identification of isolated phytochemicals The structures of 3 phytochemicals **1-3** were confirmed by spectroscopic analysis and comparison with values previously reported (17). Here, we report physical and spectral data to determine their structures (Fig. 1).

5,7-Dihydroxychromone (1): Pale yellow prisms; mp 279-280°C (19); EIMS *m/z* (relative intensity) 178 (M^+, 100), 152 (15), 124 (18); IR (KBr) ν_{max}, 3420, 1640/cm; UV λ_{max} 222, 254 nm (MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.20 (1H, d, J=2.0 Hz, H-6), 6.28 (1H, d, J=6.0 Hz, H-3), 6.37 (1H, d, J=2.0 Hz, H-8), 8.19 (1H, d, J=6.0 Hz, H-2), and 12.71 (1H, s, 5-OH). ^{13}C NMR (125 MHz, DMSO-d_6): see Table 1.

Eriodictyol (2): Slightly yellow powder; mp 265-267°C (19); EIMS *m/z* (relative intensity) 288 (M^+ 83), 179 (40), 166 (100); IR (KBr) ν_{max} 3,340, 1,650, 1,550/cm; UV λ_{max} 254, 280 nm (MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.69 (1H, dd, J=3.1 and 17.1 Hz, H-3a), 3.19 (1H, dd, J= 3.1 and 17.1 Hz, H-3b), 5.39 (1H, dd, J=3.0 and 12.5 Hz, H-2), 5.88 (1H, d, J=2.0 Hz, H-6), 5.89 (1H, d, J=2.0 Hz, H-8), 6.75 (2H, s, H-5' and H-6'), 6.88 (1H, s, H-2'), 9.01 (1H, s, 4'-OH), 9.05 (1H, s, 3'-OH), 10.8 (1H, br, 5-OH), and 12.1 (1H, s, 4-OH). ^{13}C NMR (125 MHz, DMSO-d_6): see Table 1.

3',4',5,7-Tetrahydroxyflavone (3): Yellowish amorphous powder; mp 317-320°C (19); EIMS *m/z* (relative intensity) 286 (M^+, 100), 153, (30), 134 (15); IR (KBr) ν_{max} 3,412, 1,645/cm; UV λ_{max} 242, 254 nm (MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.20 (1H, d, J=2.1 Hz, H-6), 6.46 (1H, d, J=2.1 Hz, H-8), 6.67 (1H, s, H-3), 6.91 (1H, d, J=8.2 Hz, H-5'), 7.41 (1H, dd, J=2.4 and 3.6 Hz, H-2'), 7.43 (1H, d, J=2.3 Hz, H-6'), 9.39 (1H, br, H-4'-OH), 9.90 (1H, br, H-3'-OH), 10.82 (1H, br, H-7-OH), and 12.97 (1H, br, H-5-OH). ^{13}C NMR (125 MHz, DMSO-d_6): see Table 1.

The structures of isolated phytochemicals **1-3** were readily identified as 5,7-dihydroxychromone (**1**), eriodictyol (**2**), and 3',4',5,7-tetrahydroxyflavone (**3**). Phytochemical **1** was obtained as pale yellow prisms and in the EIMS, the molecular ion peak showed as *m/z* 178. IR spectrum showed strong absorption bands at 3,420 and 1,608/cm, which indicated the presence of hydroxyl and carbonyl moiety, respectively. Analysis of the ^{1}H-NMR spectrum exhibited signals due to 2 aromatic *meta*-split doublets at δ 6.37 and 6.20 with J=2.0 Hz, for H-8 and H-6, respectively. Moreover, the presence of 2 olefinic protons resonating at 6.28 (d, J=6.0 Hz, H-3) and 8.19 (d, J=6.0 Hz, H-2) and a chelated hydroxyl group at 12.7 (s, 5-OH) suggested that phytochemical **1** was a chromone structure. The ^{13}C-NMR and DEPT spectrum showed the presence of 9 carbons as 4 protonated carbons [δ 158.0 (C-2), 110.9 (C-3), 99.5 (C-6), and 94.4 (C-8)], 4 quaternary carbons [δ 105.3 (C-10), 158.3 (C-9), 162.1 (C-5), and 164.8 (C-7)], and 1 carbonyl

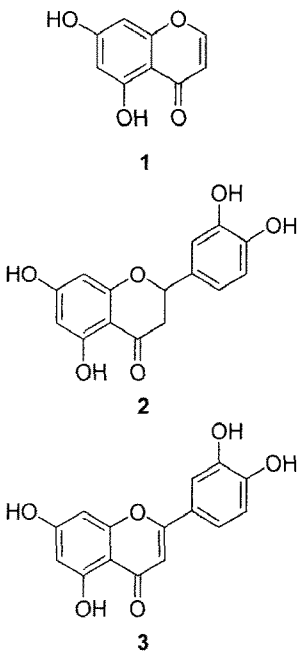

Fig. 1. Chemical structures of isolated phytochemicals 1-3 from peanut (*A. hypogaea*) pods.

Table 1. ^{13}C-NMR of phytochemicals 1-3 at 125 MHz (ppm, m)[1)]

Position	Phytochemical		
	1	2	3
1			
2	158.0 (d)	78.8 (d)	164.3 (s)
3	110.9 (d)	42.4 (t)	103.2 (d)
4	181.8 (s)	197.0 (s)	182.0 (s)
5	162.1 (s)	163.8 (s)	161.9 (s)
6	99.5 (d)	96.1 (d)	99.2 (d)
7	164.8 (s)	167.0 (s)	164.5 (s)
8	94.4 (d)	95.3 (d)	94.2 (d)
9	158.3 (s)	163.3 (s)	157.7 (s)
10	105.3 (s)	102.2 (s)	104.1 (s)
1'		129.8 (s)	121.9 (s)
2'		114.7 (d)	113.8 (d)
3'		145.6 (s)	146.1 (s)
4'		146.1 (s)	150.1 (s)
5'		115.7 (d)	116.4 (d)
6'		118.3 (d)	119.3 (d)

[1)]The chemical shifts of phytochemicals **1-3** were determined in DMSO-d_6.

carbon at δ 181.8. Confirmation of the ^{1}H- and ^{13}C-NMR shift assignments was accomplished via the HMQC spectrum, in which 1 bond proton-carbon chemical shift correlations were established, and via the HMBC spectrum in which long range hetero nuclear correlations were ascertained (Fig. 2). Therefore, the structure of phytochemical **1** was assigned as 5,7-dihydroxychromone (19). Phytochemical **2** was a slightly yellow powder and its EIMS showed a major ion peak at *m/z* 288. The IR spectrum showed the presence of hydroxyl (3,340/cm), carboxyl (1,650/cm), and aromatic C=C (1,550/cm). The ^{1}H- and ^{13}C-NMR data with DEPT experiments showed the presence of 15 carbon atoms as 6 methins [δ 78.8 (C-2), 95.3 (C-8), 96.1 (C-6), 114.7 (C-2'), 115.7 (C-5'), and 118.3 (C-6')], 1 carbonyl δ 196.7 (C-4), 7 quaternary carbons [δ 163.8 (C-5), 167.0 (C-7), 163.3 (C-9), 102.2 (C-10), 129.8 (C-1'), 145.6 (C-3'), and 146.1 (C-4')], and 1 methylene δ 42.4 (C-3). The ^{1}H-NMR spectrum showed the presence of a chelated hydroxyl group (δ 12.1, 4-OH) and an A-ring protons [δ 5.88 (1H, d, *J*=2.0 Hz, H-6) and δ 5.89 (1H, d, *J*=2.0 Hz, H-8)]. Namely, the ^{1}H-NMR spectra showed 2 *meta*-coupled doublets ascribable to H-6 and H-8 of A-ring and the presence of the flavanone skeleton was evident from ^{1}H-NMR [δ 2.69 (1H, dd, *J*= 3.1 and 17.1 Hz, H-3α) and 3.19 (1H, dd, *J*=3.1 and 17.1 Hz, H-3β)] and ^{13}C-NMR [δ 42.4 (C-3) and 196.7 (C-4)] spectra. Moreover, the substitution pattern of the B-ring was elucidated from its spectrum, showing a distinct ABX system [δ 6.88 (1H, s, H-2), δ 6.75 (2H, s, H-5' and H-6')] which confirmed the presence of a catechol ring. Also, the ^{1}H-^{1}H COSY spectrum showed correlation peak H-2–H-3 and unassigned connectivities of carbonyl and quaternary carbons were determined on the basis of HMBC correlations. The ^{1}H- and ^{13}C-long range correlation in HMBC spectrum showed cross peaks between H-2 and C-1', C-2', C-6', C-3, C-4, and C-9; between H-3 and C-1', C-3β, C-4, and C-10 (Fig. 2). Thus, these assignments and analysis of HMBC spectra allowed the unequivocal assignment of all carbons (Fig. 2). All data mentioned above indicate that the structure of phytochemical **2** was eriodictyol (28). Phytochemical **3** was also a yellowish amorphous powder and its EIMS showed a major ion peak at *m/z* 286. IR spectrum analysis exhibited strong hydroxyl and carbonyl group absorption bands at 3,412 and 1,645/cm respectively. The ^{1}H-NMR and ^{1}H and ^{13}C COSY spectrums proved the presence of 3 hydroxyl [δ 9.39 (1H, br, H-4'-OH), δ 9.90 (1H, br, H-3'-OH), and δ 10.82 (1H, br, H-7-OH)] and 1 chelated hydroxyl group [δ 12.97 (1H, br, H-5-OH)] and 6 aromatic protons. The presence of *meta*-coupled doublets at δ 6.20 (1H, d, *J*=2.1 Hz) and 6.46 (1H, d, *J*=2.1 Hz) were described to 6 and 8 protons, respectively. Furthermore, 3 protons signals at δ 6.91 (1H, d, *J*=8.2 Hz), 7.41 (1H, dd, *J*=2.4 and 3.6 Hz), 7.43 (1H, d, *J*=2.3 Hz) corresponding to 5', 2', and 6', respectively. The presence of one singlet at δ 6.67 corresponding to proton at 3 position of flavone moiety, indicate that phytochemical **3** was a flavone derivative. The connectivity of H-6' with C-2 as well as H-3 with C-1', C-2, C-4, and C-10 were determined on the basis of HMBC correlations (Fig. 2). Thus, these assignments and analysis of HMBC spectra allowed the unequivocal assignment of all carbons (Fig. 2) and all data mentioned above indicate that the structure of phytochemical **3** was 3',4',5,7-tetrahydroxyflavone (29).

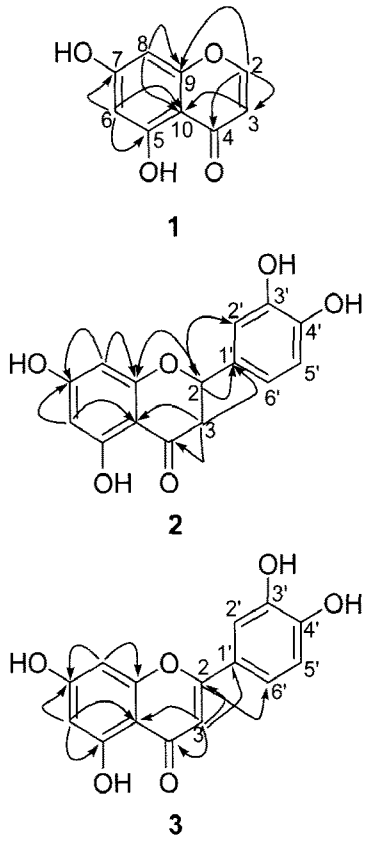

Fig. 2. Important HMBC correlations in phytochemicals 1-3.

Profile of phytochemicals The typical HPLC chromatogram of phytochemicals **1-3** are represented in Fig. 3 and retention times are as follows: 5,7-dihydroxychromone (14.1 min), eriodictyol, (26.6 min), and 3',4',5,7-tetrahydroxyflavone (29.6 min), respectively. To quantitatively analyze, calibration curves were constructed in the range 1-125 μg/mL. The regression equations of these curves and their coefficients of determination (R^2) were calculated as follow: 5,7-dihydroxychromone, y=21.86x–3.34, R^2=0.999; eriodictyol, y=36.6x–7.68, R^2= 0.997; 3',4',5,7-tetrahydroxyflavone, and y=8.57x+13.93, R^2=0.998, respectively. The method showed a linear relationship between peak areas and concentrations over this range. When the sample solutions were analyzed in the evaluated method, the peaks were identified by comparison of the retention time with those corresponding to authentic sample purified from peanut pods.

LC/MS by phytochemicals from peanut pods Three phytochemicals **1-3** were identified by comparison of their retention times and mass spectral data. For better characterization of phytochemicals, HPLC/DAD was combined with HPLC/MS operating in the positive mode at 80 eV for 5,7-dihydroxychromone (**1**), and in the negative mode at 180 eV for eriodictyol (**2**), and 3',4',5,7-tetrahydroxyflavone (**3**). As shown in Fig. 4, phytochemicals **1-3** showed the details of ions observed in the positive and negative ion spectra of different phytochemicals generated by LC/MS. Ion peak at *m/z* 178.96 was the positive

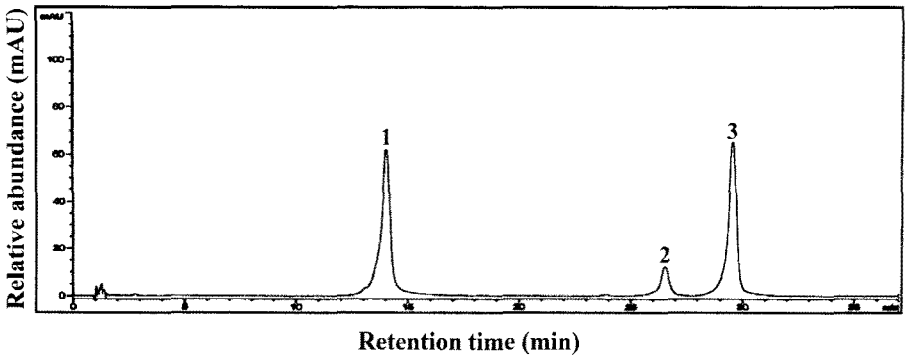

Fig. 3. HPLC chromatogram of phytochemicals. 1, 5,7-dihydroxychromone; 2, eriodictyol; and 3, 3′,4′,5,7-tetrahydroxyflavone.

molecular ion obtained for the 5,7-dihydroxychromone (**1**) (Fig. 4A), and ion peaks at *m/z* 287.10 and 284.94 were the negative molecular ion obtained for the eriodictyol (**2**) (Fig. 4B), and 3',4',5,7-tetrahydroxyflavone (**3**) (Fig. 4C). As a consequence, we can confirm their structures from their molecular ions and specific fragment ions.

Optimization of extraction conditions The pods of peanut germplasm was using the initial protocol (1.0 g, 10 mL), different solvent systems (EtOH, MeCN, and MeOH), different extraction times (1, 3, 6, 9, and 12 hr), and 2 temperature (room temperature and 50°C) by the ultrasonic bath. At first, we tested peanut germplasm under sonication using solvent including EtOH, MeCN, and MeOH for 12 hr at room temperature. The MeOH were the solvent that produced the highest yield of isolated phytochemicals (**1**: 273.71, **2**: 26.72, and **3**: 1,502.35 μg/g, respectively) (Table 2). Especially, to obtain the best MeOH extraction condition, the pods of peanut germplam extracts was obtained using solvents with 0, 10, 30, 50, 70, and 90% of water. As shown in Fig. 5 and Table 2, although, phytochemicals **2** and **3** did not showed any observable detection up to 30% MeOH extracts, phytochemical **1** exhibited 10-100% MeOH extracts. Also, the best extraction solvent for 3 phytochemicals was 90% MeOH and their contents including 5,7-dihydroxychromone (**1**), eriodictyol (**2**), and 3',4',5,7-tetrahydroxyflavone (**3**) were 407.56±33.99, 52.92±25.65, and 2,024.34±108.21 μg/g, respectively (Fig. 5E and Table 2). As shown in these results, the phytochemical concentrations of peanut pods showed that 3',4',5,7-tetrahydroxyflavone was the highest, followed by 5,7-dihydroxychromone, and eriodictyol was the lowest. The HPLC chromatogram of Fig. 5E showed a plot of the extraction efficiency of isolated phytochemical contents under the different extraction solvent systems. It was clearly necessary to add a certain amount of water to

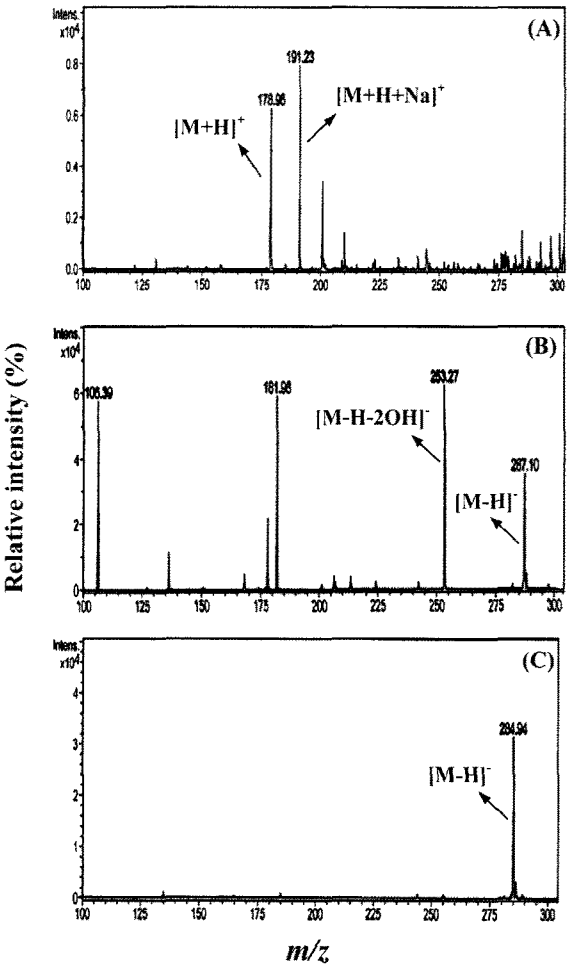

Fig. 4. Characteristic ESI mass spectra of phytochemicals 1-3. A, positive ion mass spectra of phytochemical 1; B, negative ion mass spectra of phytochemical 2; and C, negative ion mass spectra of phytochemical 3.

Table 2. Contents of 3 phytochemicals using different extraction solvents for 12 hr at room temperature[1)] (μg/g)

Phytochemical	Extraction solvent (% MeOH)					
	10	30	50	70	90	100
5,7-Dihydroxychromone (**1**)	336.81±10.79	278.52±25.97	392.94±31.93	370.92±22.12	407.56±33.99	273.71±12.56
Eriodictyol (**2**)	ND	ND	13.45±11.28	48.15±32.93	52.92±25.65	26.72±14.96
3',4',5,7-Tetrahydroxyflavone (**3**)	ND	ND	433.47±32.23	1,573.69±76.56	2,024.34±108.21	1,502.36±83.72

[1)]The values indicate the mean's of triplicate of the experiment (mean±SD, n=3); ND=not detected.

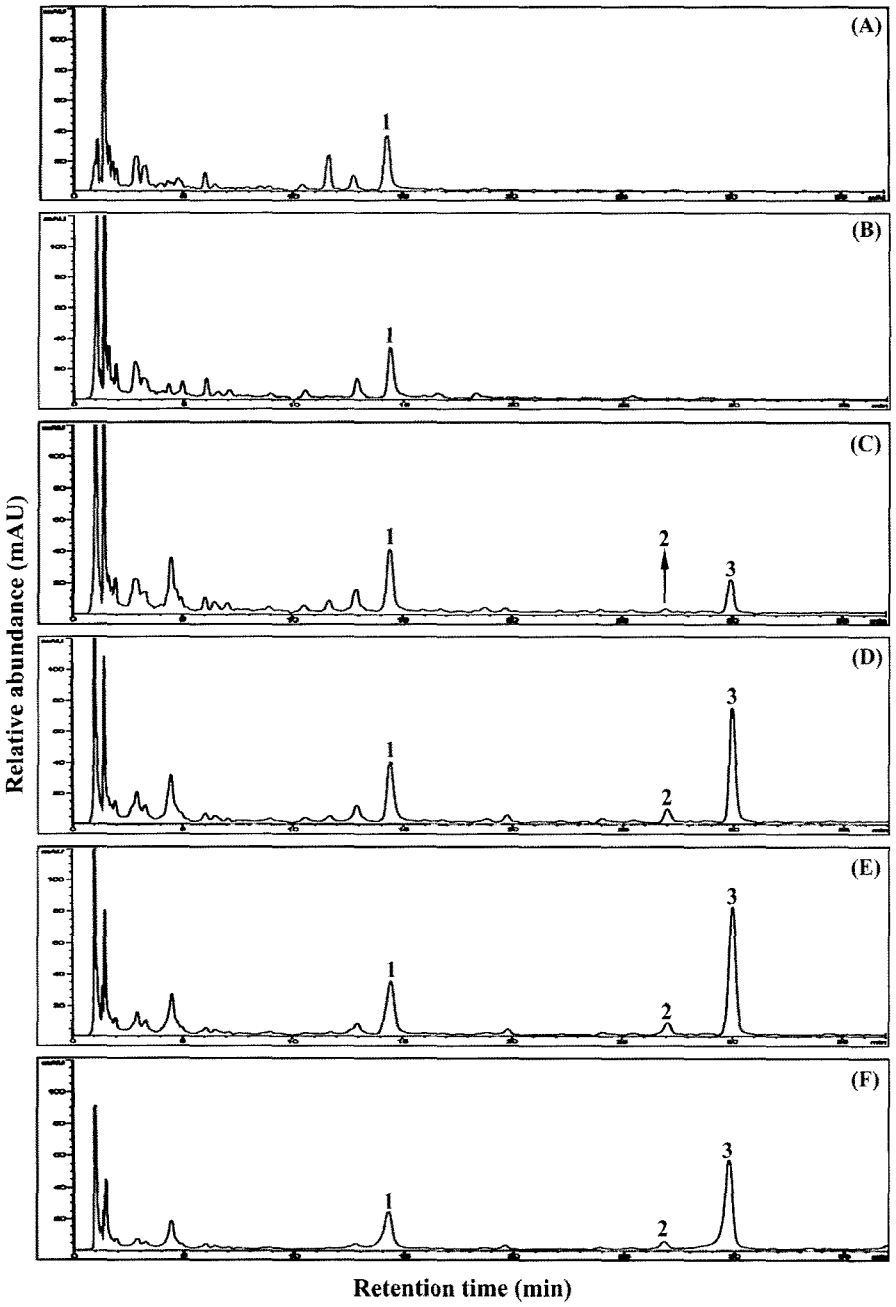

Fig. 5. HPLC chromatograms of the different solvent extracts concern to MeOH for 12 hr at room temperature. A, 10% MeOH extracts; B, 30% MeOH extracts; C, 50% MeOH extracts; D, 70% MeOH extracts; E, 90% MeOH extracts; F, 100% MeOH extracts.

the extracting solvent in order to improve the extraction of phytochemicals **1-3** from peanut pods (Fig. 5).

At second, in order to evaluate the effects of the temperature on the extraction efficiency of phytochemicals **1-3**, 90% MeOH extracts was performed at room temperature and 50°C. Although the extractions of phenolic compounds have a great impact on the temperature (30,31), as the results of this study, the effect of the temperature was not detected to improve the extraction of three phytochemicals. At third, in order to investigate the influence of extraction time on the extraction efficiency, we studied the extraction depending on 90% MeOH for 1, 3, 5, 7, 9, 12, and 15 hr by increasing extraction time at room temperature. As shown in Fig. 6, the longer the extraction times, the higher the extraction efficiency of 3 phytochemicals, up to 12 hr and then, between 12 and 15 hr, the amount extracted of phytochemicals **1-3** tested decreased slightly. Among them, 3',4',5,7-tetrahydroxyflavone (**3**) concentration significantly increased with extraction time of 5 hr, while concentrations of 5,7-dihydroxychromone (**1**) and eriodictyol (**2**) tended to slightly increased according to extraction time (Fig. 6). As evidenced from Fig. 6 and Table 3, the highest yield of phytochemicals **1-3** was obtained extraction time of 12 hr.

As a result, the optimal extraction conditions of phytochemical concentrations were obtained by employing 90% MeOH for 12 hr at room temperature.

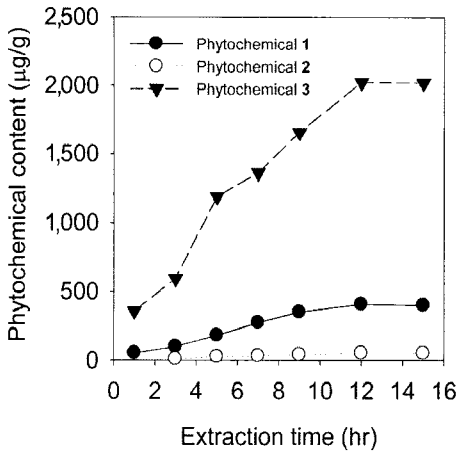

Fig. 6. Contents of phytochemicals 1-3 using 90% MeOH extracts for different extraction times at room temperature.

Comparison of phytochemical contents from the pods of peanut cultivars A typical HPLC chromatogram of 3 phytochemicals in peanut pods was shown in Fig. 3. Under optimal extraction conditions, phytochemical contents in 3 peanut cultivars grown in Miryang were shown in Table 4. There were significant differences in phytochemical contents that included 5,7-dihydroxychromone (**1**), eriodictyol (**2**), and 3',4',5,7-tetrahydroxyflavone (**3**) on the basis of HPLC peak areas with monitoring at 254 nm (Fig. 7). The highest 3',4',5,7-tetrahydroxyflavone (**3**) content was 5,162.9 ±148.64 μg/g in peanut cv. Jakwang, while the lowest was 1,338.01±62.17 μg/g in peanut cv. Daekwang. Also, the highest 5,7-dihydroxychromone (**1**) content was 1,362.10 μg/g in 'Jakwang' followed by 'Palkwang' (890.17± 30.14 μg/g). On the other hand, 'Daekwang' (590.13± 22.23 μg/g) was the lowest (Fig. 7 and Table 4) and eriodictyol (**2**) concentration was the lowest in all cultivars (25.12-186.85 μg/g).

In this work, optimization of extraction conditions of phytochemicals including 5,7-dihydroxychromone (**1**), eriodictyol (**2**), and 3',4',5,7-tetrahydroxyflavone (**3**) by extraction solvents, temperatures, and times were determined. The best condition for the phytochemicals of peanut pods was to digest the dry sample in 90% MeOH for 12 hr at room temperature. Also, the contents of phytochemical in 3 Korea cultivars using these conditions were determined. Among 3 cultivars, the highest total phytochemical content was observed in the 'Jakwang' and the lowest was in the 'Daekwang'. The results of this study suggested that the peanut pods might possess possible health related benefits to humans. In future studies, we will closely examine that further isolation of phytochemicals and evaluation of the bioactive properties.

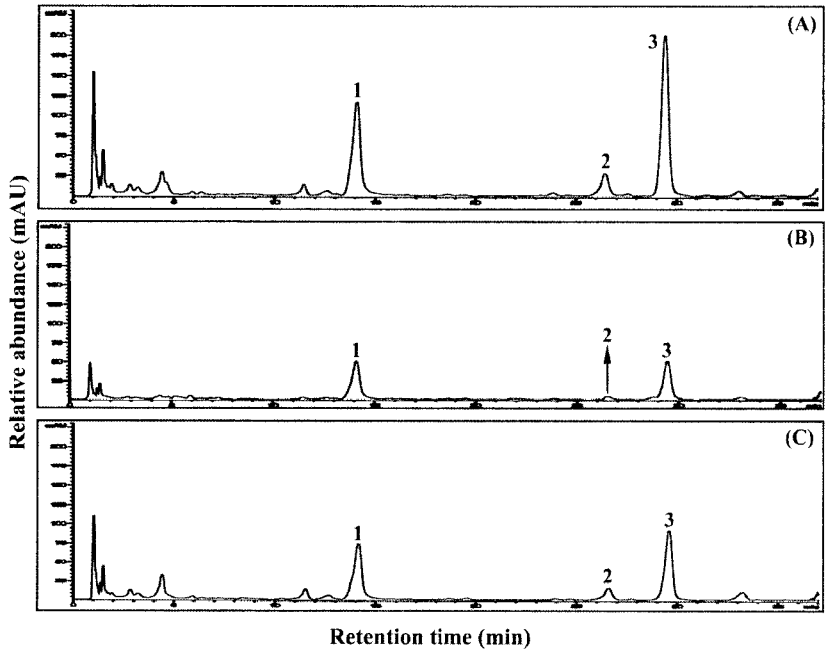

Fig. 7. Comparison of HPLC chromatograms of phytochemicals 1-3 from the pods of 3 Korea peanut cultivars. A, 'Jakwang'; B, 'Daekwang'; C, 'Palkwang'.

Table 3. Contents of 3 phytochemicals using 90% MeOH at room temperature for different extraction times[1)] (μg/g)

Phytochemical	Extraction time (hr) at 90% MeOH						
	1	3	5	7	9	12	15
5,7-Dihydroxychromone (**1**)	52.32±8.25	98.65±8.65	180.96±20.19	273.90±20.42	350.89±19.58	407.56±23.35	400.60±17.45
Eriodictyol (**2**)	ND	8.96±0.93	25.65±1.48	30.98±2.43	40.24±3.55	52.92±5.11	50.98±8.75
3',4',5,7-Tetrahydroxyflavone (**3**)	358.00±29.45	593.30±32.42	1,190.35±95.13	1,365.12±80.35	1,658.29±96.47	2,024.34±134.18	2,020.45±121.12

[1)]The values indicate the mean's of triplicate of the experiment (mean±SD, n=3); ND=not detected.

Table 4. Contents of 3 phytochemicals using 90% methanol solvent for 12 hr at room temperature from 3 cultivars

Cultivar	Contents of phytochemical (μg/g)[1)]		
	5,7-Dihydroxychromone (**1**)	Eriodictyol (**2**)	3',4',5,7-Tetrahydroxyflavone (**3**)
'Jakwang'	1,362.10±52.49	186.85±17.69	5,162.93±148.64
'Daekwang'	590.13±22.23	25.12±2.45	1,338.01±62.17
'Palkwang'	890.17±30.14	111.31±12.81	2,490.96±92.89

[1)]The values indicate the mean's of triplicate of the experiment (mean±SD, n=3).

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References

1. Milner A. Functional foods and health promotion. J. Nutr. 129: 1395-1397 (1999)
2. Silva BM, Andrade PB, Valentão P, Ferreres F, Seabra RM, Ferreira MA. Quince (*Cydonia oblonga* Miller) fruit (pulp, peel, and seed) and jam: Antioxidant activity. J. Agr. Food. Chem. 52: 4705-4712 (2004)
3. Marco DBF, Joseph V, John K. Mechanism of disease: Antioxidants and atherosclerotic heart disease. New Engl. J. Med. 337: 408-416 (1997)
4. Vinson J, Su X, Zubik L, Bose P. Phenol antioxidation quantity and quality in foods: Fruit. J. Agr. Food. Chem. 49: 5315-5321 (2001)
5. Lee JH, Baek IY, Ko JM, Kang NS, Shin SH, Lim SG, Oh KW, Shin SO, Park KY, Park KH, Ha TJ. Antioxidant and tyrosinase inhibitory activities from seed coat of brown soybean. Food Sci. Biotechnol. 17: 1-7 (2008)
6. Duh PD, Yeh DB, Yen GC. Extraction and identification of an antioxidative component from peanut hulls. J. Am. Oil Chem. Soc. 69: 814-818 (1992)
7. Huang SC, Yen GC, Chang LW, Yen WJ, Duh PD. Identification of an antioxidant, ethyl protocatechuate, in peanut seed testa. J. Agr. Food Chem. 51: 2380-2383 (2003)
8. Pratt DE, Miller EE. A flavonoid antioxidant in spanish peanuts (*Arachia hypogoea*). J. Am. Oil Chem. Soc. 61: 1064-1067 (1984)
9. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CWW, Fong HHS, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275: 218-220 (1997)
10. Reed KA, Sims CA, Gorbet DW, O'Keefe SF. Storage water activity affects flavor fade in high and normal oleic peanuts. Food Res. Int. 35: 769-774 (2002)
11. Talcott ST, Duncan CE, Del Pozo-Insfran D, Gorbet DW. Polyphenolic and antioxidant changes during storage of normal, mid, and high oleic acid peanuts. Food Chem. 89: 77-84 (2005)
12. Lee JH, Baek IY, Kang NS, Ko JM, Kim HT, Jung CS, Park KY, Ahn YS, Suh DY, Ha TJ. Identification of phenolic compounds and antioxidant effects from the exudate of germinating peanut (*Arachis hypogaea*). Food Sci. Biotechnol. 16: 29-36 (2007)
13. Hashim I, Koehler PE, Eitenmiller RR. Tocopherols in runner and Virginia peanut cultivars at various maturity stages. J. Am. Oil Chem. Soc. 70: 633-635 (1993)
14. Bland JM, Lax AR. Isolation and characterization of a peanut maturity-associated protein. J. Agr. Food Chem. 48: 3275-3279 (2000)
15. Ohno S, Tomita-Yokotani K, Kosemura S, Node M, Suzuki T, Amano M, Yasui K, Goto T, Yamamura S, Hasegawa K. A species-selective allelopathic substance from germinating sunflower (*Helianthus annuus* L.) seeds. Phytochemistry 56: 577-581 (2001)
16. Tsanuo MK, Hassanali A, Hooper AM, Khan Z, Kaberia F, Pickett JA, Wadhams LJ. Isoflavanones from the allelopathic aqueous root exudate of *Desmodium uncinatum*. Phytochemistry 64: 265-273 (2003)
17. Tian S, Nakamura K, Kayahara H. Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. J. Agr. Food Chem. 52: 4808-4813 (2004)
18. Duncan CE, Gorbet DW, Talcott ST. Phytochemical content and antioxidant capacity of water-soluble isolates from peanuts (*Archis hypogaea* L.). Food Res. Int. 39: 898-904 (2006)
19. Pendse R, Rama Rao AV, Venkataraman K. 5,7-Dihydroxy-chromone from *Arachis hypogoea* shells. Phytochemistry 12: 2033-2034 (1973)
20. Wee JH, Moon JH, Eun JB, Chung JH, Kim YG, Park KH. Isolation and identification of antioxidants from peanut shells and the relationship between structure and antioxidant activity. Food Sci. Biotechnol. 16: 116-122 (2007)
21. Gonzalez MJ, Pinto MMM, Kijjoa A, Kengthong S, Mondanondra IO, Silva AMS, Eaton G, Herz W. 5,7-Dihydroxychromones and 8-hydroxylatedhydrochromones from *Horsfieldia irya*. Phytochemisty 61: 995-998 (2002)
22. Yun BS, Lee IK, Kim JP, Chung GS, Shim GS, Yoo ID. Lipid peroxidation inhibitory activity of some constituents isolated from the stem bark of *Eucalyptus globules*. Arch. Pharm. Res. 23: 147-150 (2000)
23. Kawaii S, Tomono Y, Katase E, Ogawa K, Yano M. Antiproliferative activity of flavonoids on several cancer lines. Biosci. Biotech. Bioch. 63: 896-899 (1999)
24. Ogata S, Miyake Y, Yamamoto K, Okumura K, Taguchi H. Apoptosis induced by the flavonoid from lemon fruit (Citrus limon BURM. f.) and its metabolites in HL-60 cells. Biosci. Biotech. Bioch. 64: 1075-1078 (2000)
25. Kimata M, Inagaki N, Nagai H. Effects of luteolin and other flavonoids on IgE-mediated allergic reaction. Planta Med. 66: 25-29 (2000)
26. Zhang YH, Park YS, Kim TJ, Fang LH, Ahn HY, Hong JT, Kim Y, Lee CK, Yun YP. Endothelium-dependent vasorelaxant and antiprolifer active effects of apigenin. Gen. Pharmacol. 35: 341-347 (2000)
27. Hertog MGL, Hollman PCH, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr. Cancer 20: 21-29 (1993)
28. Geiger H, Markham KR. Campylopusaurone, an auronoflavanone biflavonoid from the mosses *Campylopus clavatus* and *Campylopus holomitrium*. Phytochemistry 31: 4325-4328 (1992)
29. Kim SJ, Cho JY, Wee JH, Jang MY, Kim C, Rim YS, Shin SC, Ma SJ, Moon JH, Park KH. Isolation and characterization of antioxidative compounds from the aerial parts of *Angelica keiskei*. Food Sci. Biotechol. 14: 58-63 (2005)
30. Kim JJ, Kim SH, Hahn SJ, Chung IM. Changing soybean isoflavone composition and concentrations under two different storage conditions over three years. Food Res. Intern. 38: 435-444 (2005)
31. Rostagno MA, Palma M, Barroso CG. Ultrasound-assisted extraction of soy isoflavones. J. Chromatogr. A 1012: 119-128 (2003)