MALDI-TOF MS Analysis of Isoflavones in Soy Products

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a new technique having a number of advantages for food analysis. This study is the first to demonstrate the use of MALDI-TOF MS to identify isoflavones in soy samples. 2′,4′,6′-Trihydroxyacetophenone (THAP) and 2,5-dihydroxybenzoic acid (DHB) were both good matrices for isoflavones, but DHB was chosen as the best because it worked well for sample extracts, with good spot-to-spot repeatability. Isoflavones were predominantly ionized in a protonated form with a very small amount of sodium or potassium adduct ions. Fragmentation occurred only through loss of glycosidic residues. Daidzin showed more than twice the response of genistin using MALDI-TOF MS. A simple solid phase extraction of isoflavones from soy samples was developed for MALDI-TOF MS analysis. MALDI-TOF MS can provide an isoflavone profile in 2 min and serves as a powerful tool to identify and study processing changes of isoflavones in soy products.

Keywords: *Soy; daidzein; genistein; glycitein*

INTRODUCTION

Soybeans or soy foods contain a very important group of compounds, isoflavones. Dietary intake of isoflavones has been associated with a low incidence of hormonally dependent and independent cancers and cardiovascular disease, reduction of menopausal symptoms such as hot flashes and osteoporosis, and prevention of hereditary chronic nose bleeds and autoimmune diseases (Messina et al., 1994; Barnes, 1998; Tham et al., 1998; Bingham et al., 1998). Soybeans contain three types of isoflavone aglycons, daidzein, genistein, and glycitein, with four chemical forms: aglycons themselves (daidzein, genistein, and glycitein); *β*-glucoside conjugates (daidzin, genistin, and glycitin); 6″-*O*-acetyl-*β*-glucoside conjugates (6″-*O*-acetyldaidzin, 6″-*O*-acetylgenistin, and 6″-*O*-acetylglycitin); and 6″-*O*-malonyl-*β*-glucoside conjugates (6″-*O*-malonyldaidzin, 6″-*O*-malonylgenistin, and 6″-*O*-malonylglycitin) (Figure 1).

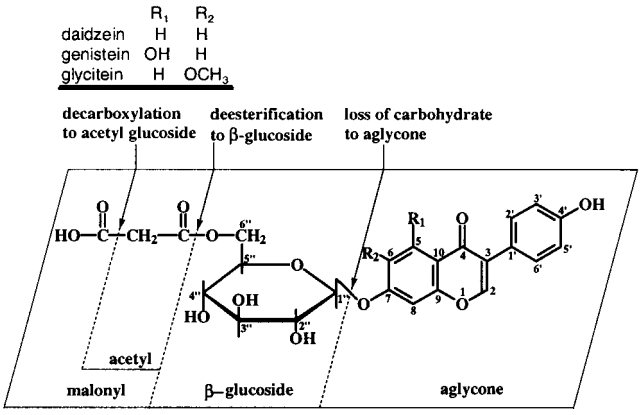

Figure 1. Chemical structures of isoflavones and possible chemical modification cleavage sites of 6″-*O*-malonyl-*β*-glucoside, 6″-*O*-acetyl-*β*-glucoside, and *β*-glucoside conjugates.

Analysis of isoflavones in food is required to help in understanding their healthy benefits or efficacy. Gas chromatography−mass spectrometry (GC-MS) is a common technique to identify and quantify isoflavones in soy or soy foods. However, only the derived aglycons can be determined (Koupai-Abyazani et al., 1992; Liggins et al., 1998; Tekel et al., 1999). High-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS), such as electrospray ionization or heated nebulizer atmospheric pressure chemical ionization, is a technique that can directly access the intact molecular weight of isoflavones, both conjugated and unconjugated (Barnes et al., 1994, 1998; Aramendia et al., 1995). HPLC is currently the most widely used analytical technique to quantify isoflavones (Murphy et al., 1997; Song et al., 1998). However, these techniques are time-consuming with tedious sample preparation.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was first introduced in 1987 and originally developed for nonvolatile and large biomolecules (Karas et al., 1987, 1988). MALDI-TOF MS has advantages over other methodologies, including speed of analysis, high sensitivity, wide applicability combined with a good tolerance toward contaminants, and the ability to analyze complex mixtures (Karas, 1996). One MALDI-TOF MS spectrum, that is, a sample profile, can be obtained in a few minutes. However, simple MALDI-TOF MS instruments cannot tell the difference between isomers, which have identical mass. The potential application of MALDI-TOF MS in food systems allows for analysis of most molecules. MALDI-TOF MS has been reported for both qualitative and quantitative analysis of anthocyanins, and flavonols, in several important foods (Wang and Sporns, 1999, 2000). This study is the first to present the use of MALDI-TOF MS to study isoflavones in soy or soy food products. The objectives of the research were to select a proper matrix for isoflavones, to study the ionization and fragmentation patterns of isoflavones in a MALDI-TOF MS system, and to develop a simple protocol for the use of MALDI-TOF MS to analyze

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isoflavones from food samples with a minimal sample purification.

MATERIALS AND METHODS

Materials and Reagents. Soybeans, tofu, and isoflavone supplements were purchased from local markets in Edmonton, AB, Canada. Soy flour A, which is different from soy flour made from the soybeans above, was obtained from our food-processing laboratory. Daidzin and genistin were obtained from Extrasynthese S.A. (Genay Cedex, France). 4-Hydroxy-α-cyanocinnamic acid (HCCA), maltotriose, and maltotetraose were purchased from Sigma Chemical Co. (St. Louis, MO). 2′,4′,6′-Trihydroxyacetophenone (THAP), 2-(4-hydroxyphenylazo)benzoic acid (HABA), 3-aminoquinoline (3-AQ), *trans*-3-indoleacrylic acid (IDA), and 2,5-dihydroxybenzoic acid (DHB) were obtained from Aldrich Chemical Co. (Milwaukee, WI). All water used was double-deionized (Milli-Q water purification system, Millipore Corp., Bedford, MA).

Extraction of Isoflavones from Soy or Soy Foods. Tofu was freeze-dried. Soybeans, freeze-dried tofu, and isoflavone supplements were pulverized using a coffee grinder (Braun Inc., Woburn, MA) to obtain a homogeneous powder. Soy flour samples (1 g), tofu (2 g), or isoflavone supplements (300 mg) were extracted with a mixture of 10 mL of acetonitrile and 2 mL of 0.1 N HCl and shaken with an orbital shaker (Lab-line Instruments, Melrose Park, NJ) for 1 h. Crude extracts were filtered through Whatman No. 4 filter paper. Filtrate (1 mL) was diluted with 9 mL of water. This 10 mL of solution was loaded onto a Sep-Pak C18 cartridge (Waters Corp., Milford, MA) at a flow rate of ~1 mL/min. Yellowish isoflavones were retained on the Sep-Pak C18 cartridge, which was then washed with 10 mL of water twice. Isoflavones were eluted with 2 mL of 70% methanol and then 100% methanol at a flow rate of ~1 mL/min. The yellowish 70% methanol eluent was demonstrated to contain the isoflavones. The isoflavone extracts were rapidly analyzed by MALDI-TOF MS without storage because the extracts were observed to give poorer spectra after overnight storage.

When isoflavones from soy flours were used for HPLC separation, 2 g samples were added to a mixture of 20 mL of acetonitrile and 4 mL of 0.1 N HCl and extracted for 1 h as above. Crude extracts were also filtered through Whatman No. 4 filter paper. Acetonitrile in the total filtrate was removed using a Büchi Rotavopor 461 with a water bath maintained at 35 °C (Brinkmann Instruments Ltd., Mississauga, ON). Acetonitrile-free filtrate (~3 mL) was loaded onto a Sep-Pak C18 cartridge (Waters Corp., Milford, MA) at a flow rate of ~1 mL/min, washed with 10 mL of water twice, and eluted with 2 mL of 70% methanol. This eluent was used for HPLC separation.

MALDI-TOF MS. MALDI-TOF MS was performed using a Proflex III in linear mode (Bruker Analytical Systems Inc., Billerica, MA). Isoflavones cocrystallized with matrices on the probe were ionized by a nitrogen laser pulse (337 nm) and accelerated under 20 kV with time-delayed extraction before entering the time-of-flight mass spectrometer. For THAP, HABA, and 3-AQ, the preparation of matrix and sample was the same as previously reported (Wang et al., 1999). The preparation of DHB and sample was as follows: the matrix solution contained 10−15 mg/mL DHB in 10% ethanol. The ratio of matrix solution to isoflavone extracts (or HPLC fractions) was 1:1. The mixture (1.5 μL) was applied to a MALDI-TOF MS probe and air-dried. MALDI-TOF MS was attenuated (the lower the attenuation, the higher the laser strength) to obtain the best signal-to-noise ratio and isotopic resolution for the matrix examined. MALDI-TOF MS was calibrated with a two-point external calibration using [maltotriose + K]$^+$ (exact isotopic mass = 543.13) and [maltotetraose + K]$^+$ (exact isotopic mass = 705.19) as calibrants, resulting in a mass accuracy of ≤500 ppm. A single spectrum was obtained by collecting 40 laser pulses from three randomly selected spots. Thus, one spectrum represented the sum of 40 pulses × 3 positions or a total of 120 laser pulses.

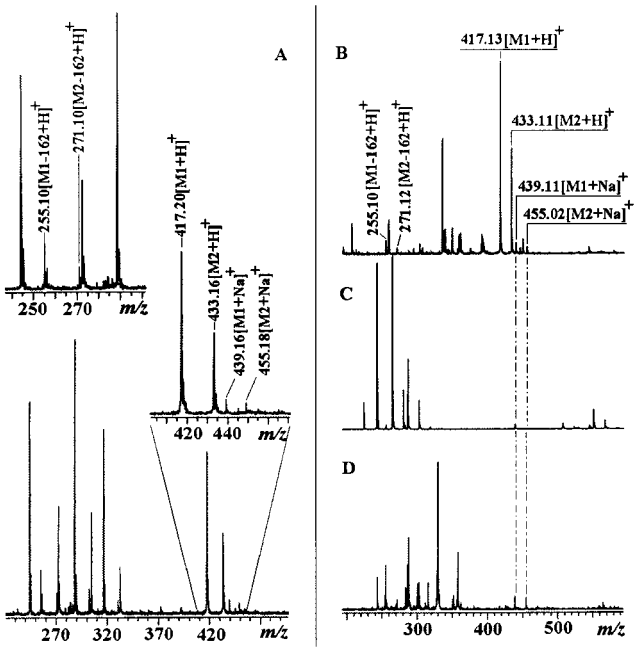

Figure 2. MALDI-TOF MS positive ion spectra of isoflavones and the performance of four MALDI-TOF MS matrices (A, DHB; B, THAP; C, HABA; D, 3-AQ) for isoflavones. M1, daidzin (6.3×10^{-4} M in 70% methanol); M2, genistin (6.7×10^{-4} M in 70% methanol).

Preparative HPLC. The preparative HPLC system consisted of a Varian VISTA 5500 HPLC (Varian Canada Inc., Mississauga, ON), a Varian 9090 autosampler, and a Spectro Monitor III UV detector (LDC/Milton Roy, Riviera Beach, FL). The system was equipped with a 75 × 4.5 mm preinjection C18 saturator column containing silica-based packing (12 μm) and a 50 × 4.6 mm guard column containing Supelco LC-18 reverse phase packing (20−40 μm, Supelco, Bellefonte, PA). Isoflavones were separated on a Supelcosil SPLC-18-DB 250 × 10 mm (5 μm) preparative reverse phase column (Supelco). The solvents used were 0.1% (v/v) aqueous acetic acid (solvent A) and acetonitrile (solvent B). The flow rate was at 5 mL/min, with a linear gradient profile consisting of solvent A with the following proportions (v/v) of solvent B: 0−1 min, 15% B; 1−50 min, 15−35% B; 50−57 min, 35% B; 57−58 min, 35−15% B; 58−60 min, 15% B. Detection was at 254 nm, and the total run time was 60 min. Sample extracts (200 μL) were injected into the HPLC for preparative collection. A Shimadzu CLASS-VP chromatography data system (Shimadzu Scientific Instruments Inc., Columbia, MD) was used to monitor the eluted peaks, and the isoflavone fractions of interest were manually collected for MALDI-TOF MS analysis.

Statistics. Means and standard deviations were analyzed using Microsoft Excel 97 (Microsoft Office 97).

RESULTS AND DISCUSSION

MALDI-TOF MS can be used to analyze most compounds in foods. However, for the compounds of interest the selection of a proper matrix in combination with various methods of sample preparation is very important. In addition, good signal-to-noise ratios and spot-to-spot repeatability are essential to acquiring a good MALDI-TOF MS spectrum for data analysis.

Performance of MALDI-TOF MS Matrices for Isoflavones. Six common matrices were examined for their performance of desorption/ionization of isoflavones. Daidzin and genistin were used as model analytes to select suitable matrices. HCCA and IDA could not generate molecular ions for daidzin and genistin, and 3-AQ (Figure 2D) and HABA (Figure 2C) produced very

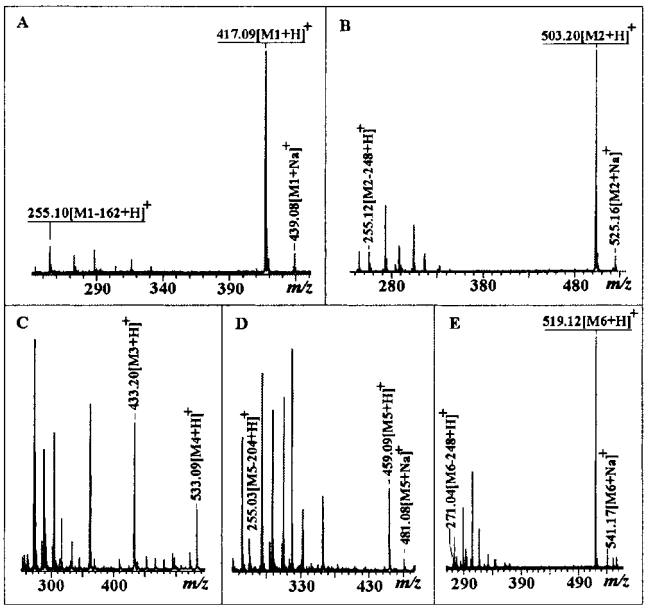

Figure 3. MALDI-TOF MS positive ion spectra of isoflavones (from soy flour A) after HPLC separation using DHB: (A) peak 1 (10.24 min), M1, daidzin; (B) peak 2 (16.92 min), M2, 6"-*O*-malonyldaidzin; (C) peak 3 (17.87 min), M3, genistin, and M4, 6″-*O*-malonylglycitin; (D) peak 4 (23.86 min), M5, 6″-*O*-acetyldaidzin; (E) peak 5 (24.55 min), M6, 6″-*O*-malonylgenistin.

low intensity molecular ions as sodium adducts. THAP (Figure 2B) and DHB (Figure 2A) produced good quality spectra in MALDI-TOF MS. Daidzin and genistin were mainly ionized as a protonated form, with very little sodium or alkali adduct ions. Compared to DHB, THAP was easier to prepare, with good spot-to-spot repeatability, and generated abundant molecular ions for the daidzin and genistin standards. However, when DHB and THAP were used with crude isoflavone extracts, DHB exhibited better performance than THAP. For the same isoflavone crude extracts, DHB generated abundant isoflavone molecular ions with a clean baseline, whereas THAP produced a poor isoflavone signal spectrum with a lot of matrix peaks (spectra not shown). Therefore, DHB was selected as the final matrix for isoflavones. Note that, in most of the figures in this paper, sodium or alkali adduct peaks were not labeled because of their low peak intensities. In all experiments, isotopic resolution was achieved for all matrices. The best spectra were those that minimized fragmentation while maintaining isotopic resolution.

Fragmentation and Responses of Isoflavones. In MALDI-TOF MS, isoflavones exhibited only fragmentation corresponding to loss of their carbohydrate residues. Figure 2 shows that daidzin and genistin fragmented and produced $[M - 162 + H]^+$ ions at *m*/*z* 255 and 271, respectively. 6″-*O*-Acetyl-*β*-glucoside and 6″-*O*-malonyl-*β*-glucoside conjugates also fragmented with glucosidic cleavage to generate their corresponding aglycons (Figure 3). This fragmentation pattern is the same as observed in electrospray ionization mass spectrometry (Barnes et al., 1994, 1998) but is different from that of heated nebulizer atmospheric pressure chemical ionization, which produces fragments from decarboxylation (Barnes et al., 1994, 1998). The fragmentation patterns of isoflavones in MALDI-TOF MS not only provided characteristic structural information of isoflavones but also were important in the study of chemical modifications of isoflavones, as discussed below.

Table 1. Quantitative Fragmentation and Responses of Isoflavones in MALDI-TOF MS

	MALDI-TOF MS sample[a]					inter-average[e]	**inter-SD**[f]
	1	2	3	4	5		
DHB as Matrix							
daidzin[b]	36.7	25.7	25.1	24.2	31.6	28.6	**5.4**
genistin[b]	39.8	28.4	25.3	23.0	29.1	29.1	**6.5**
intra-average[c]	38.2	27.0	25.2	23.6	30.3		
intra-SD[d]	**2.2**	**1.9**	**0.2**	**0.9**	**1.8**		
response ratios[g]	3.4	2.0	2.0	2.2	3.7	2.6	0.8
THAP as Matrix							
daidzin[b]	4.9	7.1	26.3	11.8	13.5	12.7	**8.4**
genistin[b]	6.6	6.3	27.3	13.1	11.0	12.9	**8.6**
intra-average[c]	5.8	6.7	26.8	12.5	12.3		
intra-SD[d]	**1.2**	**0.6**	**0.7**	**0.9**	**1.7**		
response ratios[g]	2.3	1.9	2.6	2.5	2.6	2.4	0.3

[a] Quantitative fragmentation (%) was expressed as ratios of the fragment ions (loss of glucoside) to their unfragmented protonated and sodium adduct molecular ions. Number of laser pulses was 3 × 40 or a total of 120 for each MALDI-TOF MS sample. [b] Concentration of daidzin or genistin in the mixture was 6.3×10^{-4} or 6.7×10^{-4} M, respectively. [c] Average of individual isoflavones within a single MALDI-TOF MS sample (in columns). [d] Standard deviation of individual isoflavones within a single MALDI-TOF MS sample (in columns). [e] Average of each isoflavone from five MALDI-TOF MS samples (in rows). [f] Standard deviation of each isoflavone from five MALDI-TOF MS samples (in rows). [g] Response ratios of all ions daidzin/genistin.

The relative fragmentation of isoflavone conjugates was examined in a quantitative manner. Isoflavones with different aglycons, for example, daidzin and genistin, were used to prepare a MALDI-TOF MS sample so that molecular or fragment ions would not overlap. The fragmentation of individual isoflavone conjugates was calculated on the basis of all the fragment ions (loss of carbohydrate residue) to their parent ions (total of unfragmented protonated and sodium adduct ions) in terms of a percentage observed in a single MALDI-TOF MS sample or MALDI-TOF MS spectrum (Table 1). Two matrices, DHB and THAP, were used to study the quantitative aspect of fragmentation. The fragmentation of isoflavone conjugates varied widely from sample to sample as indicated by large interstandard deviation (last column, Table 1). Within any single MALDI-TOF MS sample, all of the isoflavone conjugates exhibited a similar fragmentation percentage with small intra-standard deviation (fourth and ninth data rows, Table 1). It seemed that DHB produced more fragment ions than THAP. In general, fragmentation patterns and amounts seemed predictable for any defined sample preparation method. The response of daidzin in MALDI-TOF MS was more than twice that of genistin (Table 1). Therefore, any isoflavone MALDI-TOF MS spectrum should provide both qualitative and quantitative profile about the isoflavones in a given sample. It should be noted that the same observation was found for flavonol glycosides and anthocyanins (Wang and Sporns, 2000; Wang et al., 2000).

Solid Phase Extraction of Isoflavones for MALDI-TOF MS. MALDI-TOF MS is a technique showing high tolerance toward contaminants because analytes of interest in a crude sample extract can often be determined by MALDI-TOF MS directly without any purification. This is likely due to the selectivity of the matrix for certain analytes. However, foods often contain many compounds that can affect ionization. Partial purification is therefore sometimes necessary. When isoflavone acetonitrile crude extracts were applied to MALDI-TOF MS for analysis, a group of compounds in the extracts,

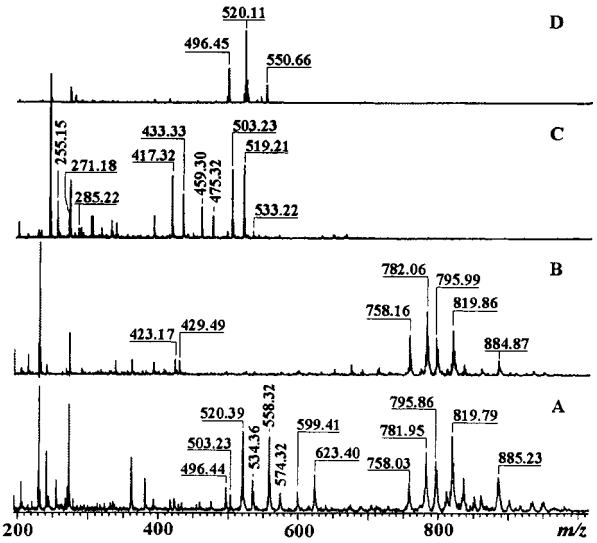

Figure 4. MALDI-TOF MS positive ion spectra of isoflavones from soy flour using DHB: (A) crude isoflavone extract; (B) water eluent passing through Sep-Pak C18 cartridge; (C) 70% methanol eluent with isoflavones from Sep-Pak C18 cartridge; (D) 100% methanol eluent from Sep-Pak C18 cartridge after 70% methanol eluent. In spectrum C, masses between 255 and 534 represent 255.15 = [daidzein + H]$^+$, 271.18 = [genistein + H]$^+$, 285.22 = [glycitein + H]$^+$, 417.32 = [daidzin + H]$^+$, 433.33 = [genistin + H]$^+$, 459.30 = [6″-*O*-acetyldaidzin + H]$^+$, 475.32 = [6″-*O*-acetylgenistin + H]$^+$, 503.23 = [6″-*O*-malonyldaidzin + H]$^+$, 519.21 = [6″-*O*-malonylgenistin + H]$^+$, and 533.22 = [6″-*O*-malonylglycitin + H]$^+$.

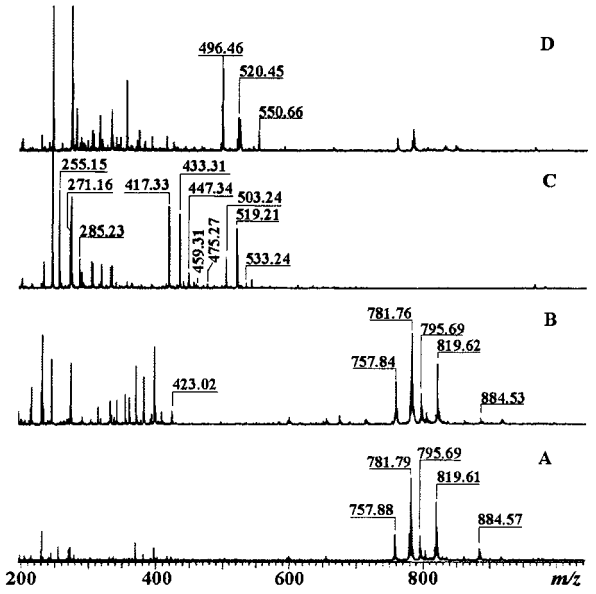

Figure 5. MALDI-TOF MS positive ion spectra of isoflavones from tofu using DHB: (A) crude isoflavone extract; (B) water eluent passing through Sep-Pak C18 cartridge; (C) 70% methanol eluent with isoflavones from Sep-Pak C18 cartridge; (D) 100% methanol eluent from Sep-Pak C18 cartridge after 70% methanol eluent. In spectrum C, masses between 255 and 534 represent 255.15 = [daidzein + H]$^+$, 271.16 = [genistein + H]$^+$, 285.23 = [glycitein + H]$^+$, 417.33 = [daidzin + H]$^+$, 433.31 = [genistin + H]$^+$, 447.34 = [glycitin + H]$^+$, 459.31 = [6″-*O*-acetyldaidzin + H]$^+$, 475.27 = [6″-*O*-acetylgenistin + H]$^+$, 503.24 = [6″-*O*-malonyldaidzin + H]$^+$, 519.21 = [6″-*O*-malonylgenistin + H]$^+$, and 533.24 = [6″-*O*-malonylglycitin + H]$^+$.

with masses between 700 and 900, seemed to suppress isoflavone ionization (Figures 4A and 5A). Some compounds, in the mass range of 450−600, might also interfere with or suppress isoflavone ions (Figure 4A). Therefore, sample purification was needed before an extract was applied to MALDI-TOF MS to generate only isoflavone ions.

Thus, a simple solid phase extraction protocol, using a Sep-Pak C18, was developed. First, the isoflavone acetonitrile crude extracts were diluted 10-fold. Then the water-based diluted extracts from above were loaded on a Sep-Pak C18 cartridge. Isoflavones were retained on the Sep-Pak C18, while the compounds with masses of 758, 782, 796, 820, and 885 passed through with water-based solvent (Figures 4B and 5B). After washing with water twice, isoflavones were eluted with 70% methanol. This eluent could be applied to MALDI-TOF MS to acquire the isoflavone spectrum of interest (Figures 4C, 5C, or 6). The final washing examined was a 100% methanol eluent that contained compounds with masses of 496, 520, and 550 (Figures 4D and 5D). The compounds with masses of 496 and 520 were from the samples because they were also observed in many crude sample extracts (Figure 4 and spectra not shown). The compound with a mass of 550 might have come from the Sep-Pak C18 cartridge because it was observed in the 100% methanol washing solution of a Sep-Pak C18 before any sample had been loaded (spectrum not shown; observed masses were 495, 523, and 550).

The recovery of the Sep-Pak C18 solid phase extraction for isoflavones was not examined in this study. However, Klejdus et al. (1999) studied all sorbents for isoflavones. They found that the recoveries of daidzin and genistin were between 84 and 88% when a C18 sorbent was used.

HPLC Separation and MALDI-TOF MS Identification of Isoflavones. After HPLC separation, seven peaks from soy flour A were collected and analyzed by MALDI-TOF MS. The relative retention time and peak positions were close to those reported by Murphy et al. (1997) and Song et al. (1998) except 6″-*O*-malonyldaidzin eluted earlier than genistin and genistin and 6″-*O*-malonylglycitin coeluted. This might be due to the pH difference of the mobile phase, solvent B. We used acetonitrile as solvent B, whereas Murphy et al. (1997) and Song et al. (1998) utilized 0.1% acetic acid in acetonitrile as solvent B. Barnes et al. (1998) indicated that the changes of pH in the mobile phase would effect the elution order of isoflavones in an HPLC system. Nevertheless, on the basis of HPLC retention time and MALDI-TOF MS characteristic ions (Figure 3), five of seven peaks were assigned to daidzin (HPLC peak at 10.24 min), 6″-*O*-malonyldaidzin (HPLC peak at 16.92 min), genistin or 6″-*O*-malonylglycitin (HPLC peak at 17.87 min), 6″-*O*-acetyldaidzin (HPLC peak at 23.86 min), and 6″-*O*-malonylgenistin (HPLC peak at 24.55 min), respectively. As expected, the fragmentation patterns included only glycosidic cleavage for the β-glucoside (Figure 3A), 6″-*O*-malonyl-β-glucoside (Figure 3B,E), or 6″-*O*-acetyl-β-glucoside (Figure 3D) in MALDI-TOF MS. No fragment ion was generated through decarboxylation and de-esterification in MALDI-TOF MS. Thus, a MALDI-TOF MS spectrum can provide the real distribution of β-glucoside, 6″-*O*-malonyl-β-glucoside, or 6″-*O*-acetyl-β-glucoside conjugates in a sample. For further confirmation, isoflavones from soybeans were also examined using the above analytical procedures, with the same results.

MALDI-TOF MS Isoflavone Profiles from Food Samples. The principal chemical forms of isoflavones in soybeans are their 6″-*O*-malonyl-β-glucoside conju-

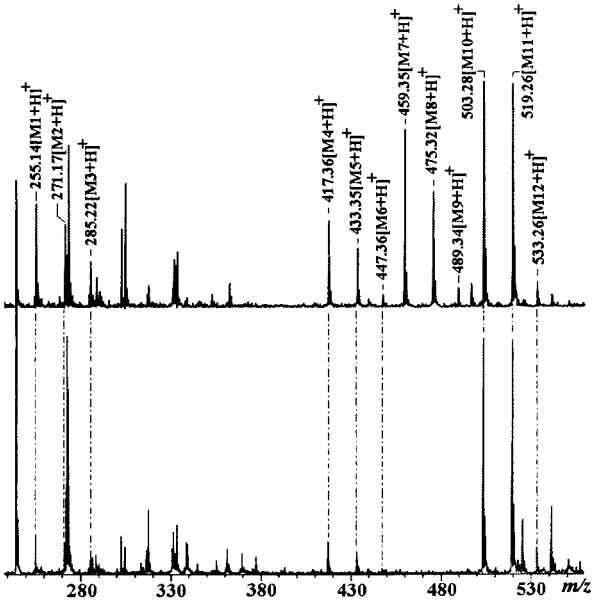

Figure 6. MALDI-TOF MS positive ion spectra of isoflavones from soybean flour using DHB: (bottom) isoflavones from original soybean flour; (top) isoflavones from soybean flour subjected to heat treatment in an oven at 105 °C for 2 h. Isoflavones were extracted using the developed solid phase extraction procedure. M1, daidzein; M2, genistein; M3, glycitein; M4, daidzin; M5, genistin; M6, glycitin; M7, 6″-*O*-acetyldaidzin; M8, 6″-*O*-acetylgenistin; M9, 6″-*O*-acetylglycitin; M10, 6″-*O*-malonyldaidzin; M11, 6″-*O*-malonylgenistin; M12, 6″-*O*-malonylglycitin.

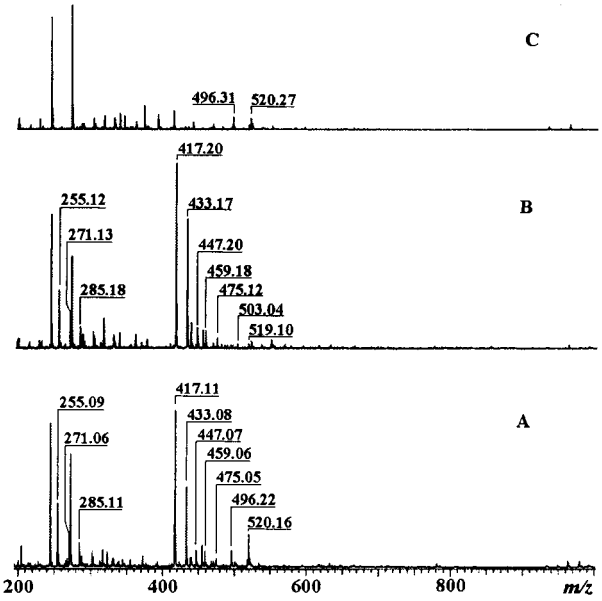

Figure 7. MALDI-TOF MS positive ion spectra of isoflavones from an isoflavone supplement using DHB: (A) crude isoflavone extract; (B) 70% methanol eluent with isoflavones from Sep-Pak C18 cartridge; (C) 100% methanol eluent from Sep-Pak C18 cartridge after 70% methanol eluent. In spectrum B, masses between 255 and 534 represent 255.12 = [daidzein + H]$^+$, 271.13 = [genistein + H]$^+$, 285.18 = [glycitein + H]$^+$, 417.20 = [daidzin + H]$^+$, 433.17 = [genistin + H]$^+$, 447.20 = [glycitin + H]$^+$, 459.18 = [6″-*O*-acetyldaidzin + H]$^+$, 475.12 = [6″-*O*-acetylgenistin + H]$^+$, 503.04 = [6″-*O*-malonyldaidzin + H]$^+$, and 519.10 = [6″-*O*-malonylgenistin + H]$^+$.

gates (Kudou et al., 1991; Coward et al., 1998). Almost all isoflavones (97−98%) in whole soybeans or other high soy protein products were in the conjugated forms, the β-glucoside, the 6″-*O*-malonyl-β-glucoside, or the 6″-*O*-acetyl-β-glucoside (Wang and Murphy, 1994). The original 6″-*O*-malonyl-β-glucoside conjugates are converted to other chemical forms of isoflavones by the action of food processing and/or fermentation. Normal heat processing causes the conversion of 6″-*O*-malonyl-β-glucoside to 6″-*O*-acetyl-β-glucoside conjugates through decarboxylation or the β-glucoside through deesterification. Fermentation produces unconjugated isoflavone aglycons as a result of fungal enzymatic β-glucosidic hydrolysis (Figure 1). Soy foods such as soy flour, tofu, and soy protein concentrate contain mainly β-glucoside conjugates, whereas fermented soyfoods such as miso and tempeh contain mainly the aglycons (Barnes et al., 1994, 1998; Wang and Murphy, 1996; Coward et al., 1998).

Figure 6 (bottom spectrum) shows the isoflavone profile from soybeans. As expected, most isoflavones are in the 6″-*O*-malonyl-β-glucoside form, with little of the β-glucosides because the sample was subjected to minimal processing. Figure 6 (top spectrum) indicates the change in isoflavones as the sample was heated in an oven at 105 °C for 2 h. Chemical modifications, that is, decarboxylation and deesterification, during heat processing are easily observed in the MALDI-TOF MS spectra (Figure 6).

Figure 7 shows the isoflavone spectra from an isoflavone supplement. The isoflavone profile from this crude sample extract (Figure 7A) is very similar to that of the sample purified by solid phase extraction (Figure 7B). The only interfering compounds were at *m*/*z* 496.31 and 520.27 (Figure 7C). This also illustrates that the solid phase extraction procedure did not change the isoflavone profile in a sample.

Because soybeans originally contain mainly 6″-*O*-malonyl-β-glucoside conjugates, as observed in Figures 4−7, MALDI-TOF MS can easily study the changes in isoflavones due to processing or can serve as a rapid analytical tool for authenticity.

In conclusion, DHB is a good matrix for the study of isoflavones using MALDI-TOF MS. Isoflavones were predominantly ionized through protonation. The fragment ions, by glucosidic cleavage of isoflavones, provided characteristic information for structural elucidation. Fragmentation and amounts of isoflavones were predictable in a MALDI-TOF MS sample. MALDI-TOF MS spectra, therefore, could provide isoflavone quantitative profiles for food samples and also be used to identify isoflavones in conjunction with other separation techniques such as HPLC.

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