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Identification of Flavonoids in Hakmeitau Beans (*Vigna sinensis*) by High-Performance Liquid Chromatography–Electrospray Mass Spectrometry (LC-ESI/MS)

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Liquid chromatography coupled with electrospray mass spectrometry (LC-ESI/MS) with positive and negative ion detection was used for the identification of flavonoids in Hakmeitau beans, a black seed cultivar of cowpea (*Vigna sinensis*). Gradient elution with water and acetonitrile, both containing 2% formic acid, was employed in chromatographic separation. The peaks were identified by comparison of the retention times and the UV−vis spectroscopic and mass spectrometric data with authentic standards and/or literature data. The identified flavonoids included six anthocyanins (cyanidin 3-*O*-galactoside, cyanidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, and petunidin 3-*O*-glucoside) and four flavonol/flavonol glycosides (kaempferol 3-*O*-glucoside, quercetin, quercetin 3-*O*-glucoside, and quercetin 3-*O*-6″-acetylglucoside). The tentatively identified flavonoids included two anthocyanins (malvidin 3-*O*-acetylglucoside and peonidin 3-*O*-malonylglucoside) and three flavonol glycosides (myricetin-3-*O*-glucoside, quercetin 7-*O*-glucoside, and quercetin-3-*O*-diglucoside). These flavonoids are present in seed coats, and the contents of anthocyanins and flavonol glycosides were 20.7 and 2.0 mg/g, respectively.

KEYWORDS: Flavonoids; anthocyanins; flavonols; glycosides; *Vigna sinensis*; bean; LC-ESI/MS

INTRODUCTION

Legumes are next to cereals in terms of their economic and nutritional importance as food resources. More studies have now revealed the beneficial or protective effects of consuming legume seeds with regard to hypercholesterolemia, cardiovascular diseases, and cancers (*1*−*3*). It is believed that the major active dietary constituents attributed to these protective effects are flavonoids (*4*−*8*). The health-related properties of flavonoids are assumed to be based on their antioxidant activities (*7*−*10*).

Our laboratory is involved in the study of nutritional values of Asiatic grain legumes and their potential food and pharmacological applications (*1*, *11*, *12*). In a screening study on the antioxidant activities of edible legume seeds, Hakmeitau bean, a black seed cultivar of cowpea (*Vigna sinensis*), was found to have the highest antioxidant activity among 24 legumes examined (*13*). Hakmeitau beans are one of the major grain legumes cultivated in Southeast Asia, and they are commonly used in soup recipes. Flavonoids are considered to be the major dietary antioxidant in food plants. The objective of this research was to identify the flavonoid constituents in Hakmeitau beans using high-performance liquid chromatography−electrospray ionization mass spectrometry (LC-ESI/MS).

MATERIALS AND METHODS

Flavonoid Standards and Reagents. All flavonoid standards were of HPLC grade. Cyanidin 3-*O*-galactoside, cyanidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, and petunidin 3-*O*-glucoside were obtained from Polyphenols (Sandes, Norway). Kaempferol 3-*O*-glucoside, quercetin, quercetin 3-*O*-glucoside, and quercetin 3-*O*-6″-acetylglucoside were purchased from Indofine Chemical Co., Inc. (Hillsborough, NJ). Acetonitrile (HPLC grade) and methanol (analytical grade) were obtained from Labscan (Asia) Co., Ltd. (Bangkok, Thailand). All other reagents were of analytical grade and obtained from Riedel-de Haën (Seelze, Germany). Distilled and deionized water was further purified by a Milli-Q water system (Millipore Ltd., Watford, U.K.) and used for all chromatographic analyses and sample and standard preparations. All stock solutions of flavonoid standards were prepared in methanol/water (50:50, v/v) containing 0.1% HCl at a concentration of 1 mg/mL and diluted with water to the desired concentrations.

Plant Materials. Hakmeitau beans were originally purchased from a local supplier. To avoid variation in seed quality, the bean plants were cultivated at our experimental farm, and seeds harvested were collected for subsequent experiments. This legume species was identified as a black seed cultivar of *V. sinensis* by Dr. S. Y. Hu of Harvard

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University. For seed coat preparation, the seeds were soaked in distilled water at 4 °C for 12 h, and the seed coats were removed manually and freeze-dried.

Extraction and Purification of Phenolic Compounds from Hakmeitau Beans. The phenolic compounds were extracted from the whole seeds and purified by solid-phase extraction using an absorption resin. Seeds were pulverized, and the seed powder (10 g) was extracted with 100 mL of 80% aqueous methanol containing 0.1% HCl by continuous shaking for 5 h. The methanolic extract was filtered with filter paper under reduced pressure. The seed residue was resuspended in 100 mL of the same solvent and extracted for 1 h. This extraction step was repeated twice. The combined methanolic extracts were concentrated with a rotary evaporator at 40 °C under reduced pressure to remove the methanol. The resulting solution (~40 mL) was mixed with 200 mL of water. After filtration, the aqueous solution was applied to a 23 × 2.7 cm i.d. Diaion HP-20 absorption resin column (Supelco, Bellefonte, PA), which was preconditioned by washing with methanol and then equilibrated with water. Nonphenolic impurities including sugars, amino acids, proteins, and minerals were washed out with water (500 mL). Phenolic compounds were eluted from the resin with methanol (200 mL), and the eluant was dried with a rotary evaporator under reduced pressure. The phenolics residue was redissolved in water (40 mL) and freeze-dried to give a purple fluffy phenolic extract powder (0.13 g).

HPLC Analysis. HPLC analysis was performed using a Waters 2690 separation module system (Waters Associates, Milford, MA) equipped with an autosampler and a Waters model 996 photodiode array detector. Chromatographic separation was carried out using a 150 × 2.1 mm i.d., 5 μm SymmetryShield C_{18} steel column with a 10 × 2.1 mm i.d., 3.5 μm Symmetry C_{18} guard column (Waters), operated at room temperature (23 °C). Mobile phase consisted of 2% formic acid in water (eluant A) and 2% formic acid in acetonitrile (eluant B) with a linear gradient elution at a flow rate of 0.2 mL/min. The elution program was as follows: 8−10% B (0−10 min); 10−20% B (10−35 min); 20−20% B (35−50 min); 20−45% B (50−60 min); and 45−8% B (60−80 min). The injection volume for all samples was 10 μL. The UV−vis absorption spectra of the column eluant were recorded from 200 to 600 nm.

LC-ESI/MS Analysis. LC-ESI/MS analyses were performed with the same HPLC system as described above interfaced to a Waters Micromass ZMD model mass spectrometer equipped with an ESI source. Mass spectrometric data of the column eluant were acquired in positive mode for anthocyanins and in negative mode for flavonol glycosides. Data acquisition and processing were performed using a Masslynx NT 3.5 data system (Micromass). The mass spectra were recorded in the range of *m*/*z* 150−800. Nitrogen was used both as drying gas and as nebulizing gas at flow rates of approximately 250 and 120 L/h, respectively. Source block temperature and desolvation temperature were set at 110 and 400 °C, respectively. Optimization of ionization conditions was based on the intensity of the mass signals of both protonated/deprotonated molecules and aglycon fragments, and was performed using the standards cyanidin 3-*O*-glucoside/quercetin 3-*O*-glucoside for positive/negative MS experiments, respectively. Mass parameters were optimized as follows: capillary voltage, 3.85/3.28 kV; and cone voltage, 20/52 V, for positive/negative ionization, respectively. The purified phenolic extract described above was dissolved in methanol/water (50:50, v/v) containing 0.1% HCl. The solution (10 mg/mL) was filtered by a syringe filter with a 0.2 μm PVDF membrane (Gelman Laboratory), and 10 μL was injected onto the HPLC column for HPLC-UV−vis and HPLC-ESI-MS analyses.

Acid Hydrolysis of Flavonol Glycosides. Acid hydrolysis of flavonol glycosides was performed according to the method of Nuutila et al. (*14*) with modifications. The phenolic extract was dissolved in methanol/water (50:50, v/v) containing 1.2 M HCl. The solution (10 mg/mL) was incubated at 80 °C in a water bath for 2 h and then filtered by a syringe filter. An aliquot (10 μL) of the filtrate was injected onto the HPLC column for analysis.

Determination of Anthocyanins and Flavonol Glycosides. The whole seed powder (500 mg) or seed coat preparation (200 mg) was soaked in 20 mL of 80% methanol containing 0.1% HCl and kept in an airtight capped bottle at room temperature for 2 h. The seed suspension was then incubated at 45 °C in a water bath with continuous shaking for an additional 30 min. After cooling to room temperature, the seed suspension was filtered by a syringe filter, and 10 μL of the filtered extract was injected into the HPLC for analysis. The experiment was performed in triplicate. The peak areas were used to calculate the concentrations of flavonoids in the whole seed extract or the seed coat extract by using the corresponding standard curves. Quantities of the flavonoids without authentic standards were calculated by using other standards with similar chemical structures and expressed as their equivalents.

RESULTS AND DISCUSSION

Extraction of Phenolic Compounds. For the extraction of phenolic compounds from the whole seed powder or the seed coat preparation, several solvents were tested including methanol, ethanol, 80% methanol, 80% ethanol, and 80% methanol containing 0.1% HCl. It was found that 80% methanol containing 0.1% HCl was the best solvent for the extraction of phenolic compounds because it produced the highest recovery of total phenolics from seed samples (data not shown). This is in agreement with previous results by Ju and Howard (*15*) indicating that acidified methanol was the most efficient solvent for extracting phenolic compounds.

During the concentration of seed extract that contained 0.1% HCl, the acid content of the solvent increased, and this might cause acid hydrolysis of the glycosidic compounds. Therefore, the changes of anthocyanins and flavonol glycosides were examined by comparing the contents of individual compounds in the extract before and after rotary evaporation at 40 °C under reduced pressure. The results showed no change had occurred, indicating that 0.1% HCl in the extraction solvent was not high enough to cause hydrolysis of anthocyanins and flavonol glycosides during concentration.

Optimization of LC-ESI/MS Conditions. To analyze anthocyanins by HPLC, the pH values of the mobile phase should be in the range from 1 to 2. Formic acid is generally used as acid modifier, and high proportions of this acid (5%) are used to reach this pH (*15*−*17*). In this study, several mobile phases were tested, including 0.1% trifluoroacetic acid (TFA) and 0.25, 2, and 5% formic acid in water and acetonitrile. Excellent chromatographic resolution and separation of anthocyanins was achieved using 0.1% TFA or 5% formic acid, but the strong acid or the high proportion of acid limited the ionization of both anthocyanins and flavonol glycosides. Therefore, 2% formic acid was chosen as acid modifier because it proved to be optimal for both chromatographic resolution and ion formation efficiency for these compounds. The gradient elution program used in this study allowed the separation of all seed flavonoids within 80 min for each run (**Figures 1** and **2**). For mass detection, the two classes of compounds, namely, anthocyanins and flavonol glycosides, should be separately analyzed using positive mode for the former and negative mode for the later, because they showed different sensitivities of detection when different ionization methods were used.

Identification of Seed Flavonoids. Once the optimal LC-ESI/MS conditions had been established for the compounds studied, the seed extract was analyzed by the method in the full scan mode. **Figures 1** and **2** show the HPLC-UV−vis and selected mass chromatograms of purified phenolic extract prepared from whole seeds. Identification of individual compounds was performed by comparison of LC retention times, photodiode array UV−vis spectroscopic, and ESI-MS spectrometric data (**Table 1**) with those of authentic standards or with published data. A total of 15 flavonoids were identified or tentatively identified in the seed extract, including 8 anthocyanins and 7 flavonol glycosides (**Figures 3** and **4**; **Table 1**).

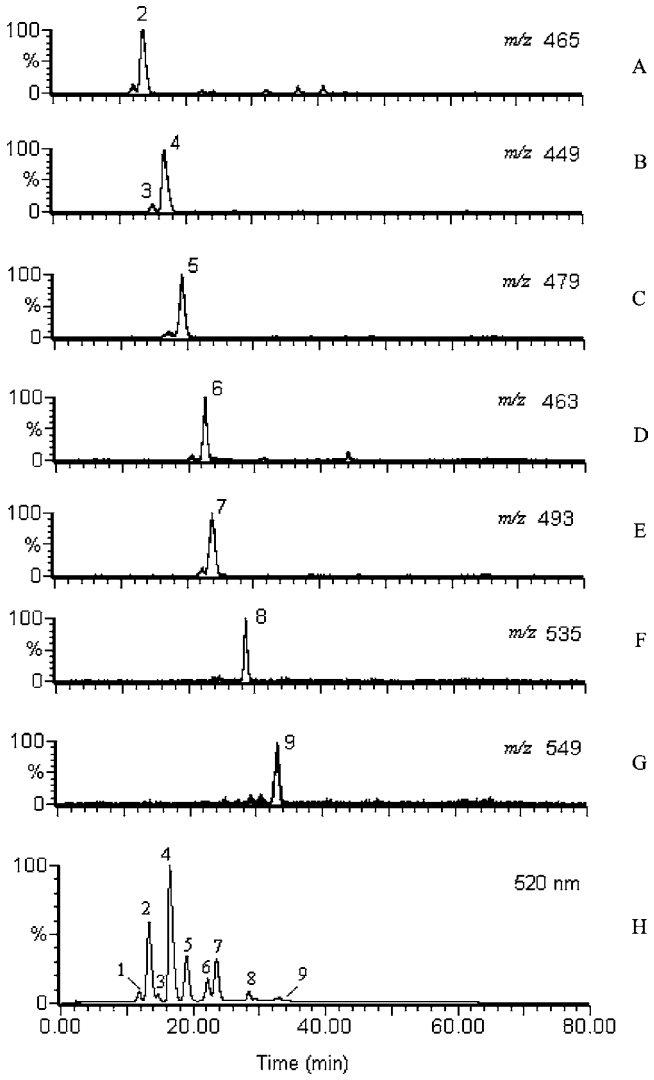


Figure 1. HPLC-vis chromatogram (520 nm) and selected mass chromatograms (in positive mode) of purified phenolic extract of Hakmeitau beans: A–G, mass chromatograms; H, HPLC chromatogram acquired at 520 nm.

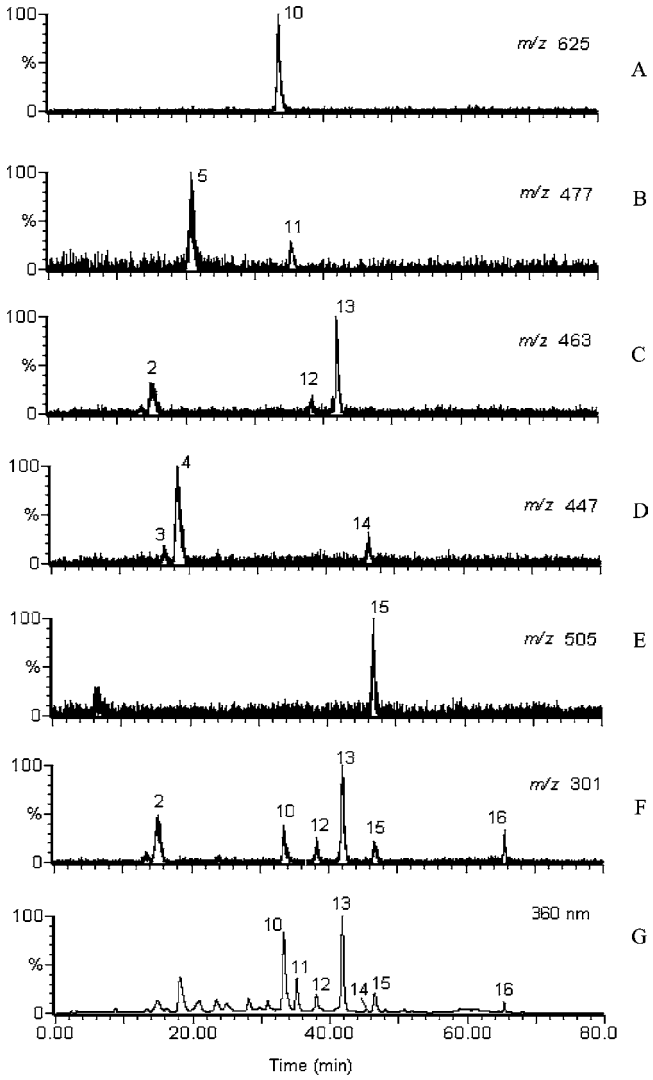


Figure 2. HPLC-UV chromatogram (360 nm) and selected mass chromatograms (in negative mode) of purified phenolic extract of Hakmeitau beans: A–F, mass chromatograms; G, HPLC chromatogram acquired at 360 nm.

Anthocyanins. In the HPLC-vis chromatogram acquired at 520 nm (**Figure 1H**), nine peaks (compounds **1**−**9**) were detected. The UV−vis spectra of these compounds all showed strong absorption at ∼520 nm, which is a characteristic of anthocyanins (*18*, *19*). In mass chromatograms selected at *m*/*z* 465, 449, 479, 463, and 493 (**Figure 1A**−**E**), six peaks (compounds **2**−**7**) were observed, the mass spectra of which showed their protonated aglycon ions $[A + H]^+$ to be *m*/*z* 303, 287, 287, 317, 301, and 331, respectively, corresponding to delphinidin, cyanidin, cyanidin, petunidin, peonidin, and malvidin (*19*). These protonated aglycon ions were all formed by loss of a sugar moiety with 162 units from their $[M + H]^+$, indicating that they are anthocyanidin monoglucosides or monogalactosides. This suggests the presence of delphinidin 3-*O*-glucoside, cyanidin 3-*O*-galactoside, cyanidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, and malvidin 3-*O*-glucoside, in addition to comparison of their retention times with those of authentic standards. For the other two anthocyanins, compounds **8** and **9**, they were observed at *m*/*z* 535 and 549 in the mass chromatograms (**Figure 1F**,**G**). Their ESI/MS data showed the $[A + H]^+$ ions at *m*/*z* 331 and 301, respectively, corresponding to malvidin and peonidin (*19*). The losses from their $[M + H]^+$ (*m*/*z* 535 and 549) to relative $[A + H]^+$ were 204 and 248, respectively, which are both larger than that of the glucosidic group. The differences of 42 and 86 correspond to acetyl group and malonyl group, respectively. Results indicated that the acetyl or malonyl group is present in the two molecules, and they should be attached to the glycosidic part of the molecule, especially in its 6-position as 6″-*O*-acetylglucoside or 6″-*O*-malonylglucoside (*20*, *21*). The glycosidic groups are most probably attached to the 3-position of the molecules, this position being the most common site for conjugation with a sugar moiety (*22*). Therefore, these two compounds were tentatively identified as malvidin 3-*O*-acetylglucoside and peonidin 3-*O*-malonylglucoside.

Flavonol Glycosides. **Figure 2G** shows the HPLC-UV chromatogram acquired at 360 nm, in which seven major peaks (compounds **10**−**16**) with typical flavonol UV spectra were observed. The maximum absorptions of these compounds were at about 265 and 360 nm, characteristic of flavonol compounds (*21*, *23*). In the *m*/*z* 625 mass chromatogram (**Figure 2A**), a peak (compound **10**) with a retention time of 33.5 min was observed, the mass spectrum of which showed a loss of *m*/*z* 324 from the deprotonated molecular ion $[M - H]^-$ of *m*/*z* 625 to the deprotonated aglycon ion $[A - H]^-$ of *m*/*z* 301, indicating the presence of quercetin and a dihexose, most probably

Table 1. Identification of Anthocyanins and Flavonol Glycosides in Hakmeitau Beans Based on HPLC Retention Time (t_R), UV–Vis Spectroscopic Characteristics (λ_{max}), and ESI/MS Spectrometric Pattern

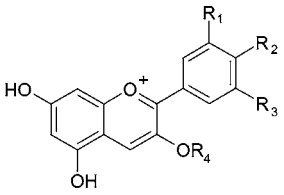
Anthocyanins

peak	t_R (min)	identified compound	λ_{max} (nm)	molecular ion $[M + H]^+$	aglycon ion $[A + H]^+$	compared with standard
1	11.9	unknown	279, 522			
2	13.3	delphinidin 3-*O*-glucoside	277, 522	465	303	yes
3	14.8	cyanidin 3-*O*-galactoside	280, 519	449	287	yes
4	16.5	cyanidin 3-*O*-glucoside	280, 514	449	287	yes
5	19.0	petunidin 3-*O*-glucoside	278, 525	479	317	yes
6	22.3	peonidin 3-*O*-glucoside	279, 518	463	301	yes
7	23.6	malvidin 3-*O*-glucoside	279, 526	493	331	yes
8	28.4	malvidin 3-*O*-acetylglucoside[a]	280, 522	535	331	no
9	32.7	peonidin 3-*O*-malonylglucoside[a]	277, 526	549	301	no

Flavonol Glycosides

peak	t_R (min)	identified compound	λ_{max} (nm)	molecular ion $[M - H]^-$	aglycon ion $[A - H]^-$	compared with standard
10	33.5	quercetin 3-*O*-glucosylglucoside[a]	265, 354	625	300, 301	no
11	35.1	myricetin 3-*O*-glucoside[a]	268, 353	477	315	no
12	38.1	quercetin 7-*O*-glucoside[a]	270, 370	463	301	no
13	41.9	quercetin 3-*O*-glucoside	267, 354	463	300, 301	yes
14	46.1	kaempferol 3-*O*-glucoside	265, 353	447	285	yes
15	46.8	quercetin 3-*O*-6″-acetylglucoside	266, 353	505	300, 301	yes
16	65.4	quercetin	277, 369	301		yes

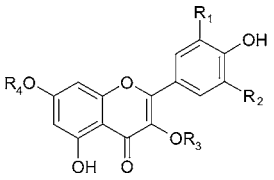
[a] Tentatively identified compounds.



Peak	Compound name	Substitution pattern R_1	R_2	R_3	R_4
2	Delphinidin-3-*O*-glucoside	OH	OH	OH	Glucose
3	Cyanidin-3-*O*-galactoside	OH	OH	H	Galactose
4	Cyanidin-3-*O*-glucoside	OH	OH	H	Glucose
5	Petunidin-3-*O*-glucoside	OMe	OH	OH	Glucose
6	Peonidin-3-*O*-glucoside	OMe	OH	H	Glucose
7	Malvidin-3-*O*-glucoside	OMe	OH	OMe	Glucose
8	Malvidin-3-*O*-acetylglucoside*	OMe	OH	OMe	Acetylglucose
9	Peonidin-3-*O*-malonylglucoside*	OMe	OH	H	Malonylglucose

* Tentatively identified compounds

Figure 3. Chemical structures of anthocyanins identified in Hakmeitau beans.



Peak	Compound name	Substitution pattern R_1	R_2	R_3	R_4
10	Quercetin-3-*O*-diglucoside*	OH	H	Glucose-Glucose	H
11	Myricetin-3-*O*-glucoside*	OH	OH	Glucose	H
12	Quercetin-7-*O*-glucoside*	OH	H	H	Glucose
13	Quercetin-3-*O*-glucoside	OH	H	Glucose	H
14	Kaempferol-3-*O*-glucoside	H	H	Glucose	H
15	Quercetin-3-*O*-6″-acetylglucoside	OH	H	6″-Acetylglucose	H
16	Quercetin	OH	H	H	H

* Tentatively identified compounds

Figure 4. Chemical structures of flavonoids identified in Hakmeitau beans.

diglucose (glucosyl-glucose) attached to the 3-position (*22*) of the molecule. No ion at *m*/*z* 463 representing the loss of a single glucose unit (162) was observed, which would be expected for either 3,5- or 3,7-disubstituted glycosides. Because the diglucoside gives three MS fragment ions including aglycon, 3-glucosylated quercetin, and 5- or 7-glucosylated quercetin, whereas the 3-diglucoside will give only a single MS fragment ion corresponding to the aglycon (*16*). In the *m*/*z* 477 mass chromatogram (**Figure 2B**), a peak (compound **11**) at 35.1 min was observed, which showed a loss of 162 from its $[M - H]^-$ to $[A - H]^-$ of *m*/*z* 315, suggesting myricetin hexoside, most probably myricetin 3-*O*-glucoside. Compounds **12** and **13** were observed in the *m*/*z* 463 mass chromatogram (**Figure 2C**) at retention times of 38.1 and 41.9 min, respectively. Both mass spectra were the same and showed the loss of 162 from the $[M - H]^-$ *m*/*z* 463 to $[A - H]^-$ *m*/*z* 301, suggesting the presence of two quercetin hexosides. By comparison with standards, compound **13** was identified as quercetin 3-*O*-glucoside and compound **12** was found not to be another quercetin hexoside, that is, quercetin 3-*O*-galactoside, due to its different retention time from the standard (41.4 min). Compound **12** is most probably quercetin 7-*O*-glucoside because the 7-position is another common site for conjugation with a sugar moiety other than the 3-position (*22*). Compounds **14**, **15**, and **16** were observed in the mass chromatograms selected at *m*/*z* 447, 505, and 301 (**Figure 2D**−**F**), respectively. The former two compounds showed losses of 162 and 204 from $[M - H]^-$ to $[A - H]^-$ of *m*/*z* 285 and 301, suggesting the presence of kaempferol glucoside and quercetin acetylglucoside, respectively. Compound **16** was found to be a flavonol, quercetin. The three compounds showed the same retention times, UV spectra, and ESI mass spectra patterns as their standards. In the *m*/*z* 301

Table 2. Contents of Anthocyanins and Flavonol Glycosides in Hakmeitau Beans As Determined by HPLC-UV–Vis[a]

compound	whole seed (mg/g)	seed coat (mg/g)
Anthocyanins		
unknown (peak 1)	0.25 ± 0.01[c]	1.02 ± 0.06[c]
delphinidin 3-*O*-glucoside	1.45 ± 0.05	7.62 ± 0.05
cyanidin 3-*O*-galactoside	0.03 ± 0.01	0.16 ± 0.01
cyanidin 3-*O*-glucoside	1.03 ± 0.04	6.14 ± 0.03
petunidin 3-*O*-glucoside	0.42 ± 0.02	2.06 ± 0.05
peonidin 3-*O*-glucoside	0.13 ± 0.01	0.77 ± 0.02
malvidin 3-*O*-glucoside	0.39 ± 0.01	2.20 ± 0.04
malvidin 3-*O*-acetylglucoside[b]	0.12 ± 0.01[d]	0.59 ± 0.01[d]
peonidin 3-*O*-malonylglucoside[b]	0.04 ± 0.01[e]	0.18 ± 0.01[e]
total	3.86 ± 0.12	20.74 ± 0.04
Flavonol Glycosides		
quercetin 3-*O*-glucosylglucoside[b]	0.12 ± 0.01[f]	0.64 ± 0.01[f]
myricetin 3-*O*-glucoside[b]	0.04 ± 0.01[f]	0.24 ± 0.01[f]
quercetin 7-*O*-glucoside[b]	0.02 ± 0.00[f]	0.12 ± 0.01[f]
quercetin 3-*O*-glucoside	0.10 ± 0.01	0.69 ± 0.01
kaempferol 3-*O*-glucoside	0.03 ± 0.01	0.19 ± 0.01
quercetin 3-*O*-6″-acetylglucoside	0.01 ± 0.00	0.05 ± 0.01
quercetin	0.01 ± 0.00	0.04 ± 0.01
total	0.33 ± 0.01	1.97 ± 0.01

[a] All values are expressed as mean ± SD of triplicates. [b] Tentatively identified compounds. [c] Expressed as delphinidin 3-*O*-glucoside equivalent [d] Expressed as malvidin 3-*O*-glucoside equivalent [e] Expressed as peonidin 3-*O*-glucoside equivalent [f] Expressed as quercetin 3-*O*-glucoside equivalent

mass chromatogram, four other peaks with retention times of 33.5, 38.1, 41.9, and 46.8 min were also detected; except for quercetin at 65.4 min, those peaks are $[A - H]^-$ of compounds **10**, **12**, **13**, and **15**, respectively. In the negative mode mass chromatograms selected at *m*/*z* 463, 447, 477, and 301 (**Figure 2B**−**D**,**F**), the anthocyanin compounds **2**, **3**, **4**, and **5** were also observed, respectively.

The aglycons from flavonol glycosides were also investigated by analyzing the phenolic extract after acidic hydrolysis. Three peaks were observed in the HPLC-UV chromatogram at 360 nm. They had flavonol UV spectra with maxima at around 253 and 370 nm and deprotonated molecular ions of *m*/*z* 315, 301, and 285, respectively, corresponding to the three aglycons, namely, myricetin, quercetin, and kaempferol. The peak area ratio of the three aglycons was 12:85:3, indicating that quercetin glycosides are the predominant flavonol glycoside in the seeds. The observation of myricetin in the hydrolyzed product provided additional evidence for the presence of myricetin 3-*O*-glucoside in the seeds.

Determination of Flavonoid Contents in Hakmeitau Beans. The contents of identified flavonoids in Hakmeitau beans were determined by HPLC-UV−vis, and the results are shown in **Table 2**. It was found that anthocyanins and flavonol glycosides were localized in the seed coats. Anthocyanins were not detected in the cotyledons (data not shown). Delphinidin 3-*O*-glucoside is a predominant anthocyanin in seed coats, with the highest content of 7.6 mg/g, followed by cyanidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, and petunidin 3-*O*-glucoside. The total contents of anthocyanins were 20.7 mg/g in seed coats and 3.9 mg/g in whole seeds. These calculations were based on the sum of all detectable anthocyanins, including peak 1, which was identified as an anthocyanin from its UV−vis spectrum (**Table 1**). Quercetin 3-*O*-glucoside and quercetin 3-*O*-diglucoside are the two major flavonol glycosides in Hakmeitau beans. The contents of total flavonol glycosides were 2.0 mg/g in seed coats and 0.3 mg/g in whole seeds.

Dietary intake of flavonoid antioxidants is shown to be related to various beneficial effects including a reduced risk of cardiovascular diseases and certain forms of cancer (*7*). With regard to flavonoids in legume seeds, most studies focus on isoflavones in soybeans, and very limited information is available on other pulses. This is the first study on the composition and contents of flavonoids in Hakmeitau beans. The high contents of flavonoids present in the seeds may contribute to the high antioxidant activity observed for Hakmeitau beans (*13*).

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