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Identification of Flavonoids and Hydroxycinnamic Acids in Pak Choi Varieties (*Brassica campestris* L. ssp. *chinensis* var. *communis*) by HPLC–ESI-MS^n and NMR and Their Quantification by HPLC–DAD

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Twenty-eight polyphenols (11 flavonoid derivatives and 17 hydroxycinnamic acid derivatives) were detected in different cultivars of the Chinese cabbage pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*) by HPLC–DAD–ESI-MS^n. Kaempferol was found to be the major flavonoid in pak choi, glycosylated and acylated with different compounds. Smaller amounts of isorhamnetin were also detected. A structural determination was carried out by ^{1}H and ^{13}C NMR spectroscopy for the main compound, kaempferol-3-*O*-hydroxyferuloylsophoroside-7-*O*-glucoside, for the first time. Hydroxycinnamic acid derivatives were identified as different esters of quinic acid, glycosides, and malic acid. The latter ones are described for the first time in cabbages. The content of polyphenols was determined in 11 cultivars of pak choi, with higher concentrations present in the leaf blade than in the leaf stem. Hydroxycinnamic acid esters, particularly malic acid derivatives, are present in both the leaf blade and leaf stem, whereas flavonoid levels were determined only in the leaf blade.

KEYWORDS: Pak choi; Chinese *Brassica* vegetables; polyphenols; flavonol glycosides; hydroxycinnamoylmalates; hydroxyferulic acid

INTRODUCTION

Pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*) is a leafy Chinese cabbage in the Brassicaceae family. Cabbages are the most important vegetables in human nutrition in China [which provided 54% of world production in 2005 (*1*)] and are mostly eaten in cooked or fermented form. Depending on climatic conditions, pak choi is cultivated in China in autumn and spring. This kind of cabbage is barely known in Europe today, but the level of consumption is expected to rise in the future.

In general, *Brassica* vegetables are known to play an important role in human nutrition due to their phytochemicals, such as vitamins, minerals, glucosinolates, and phenolic compounds (*2*–*5*). In particular, it has been shown that *Brassica* species potentially exert inhibitory activity against chronic diseases like cancer (*6*). Phenols such as flavonoids and hydroxycinnamic acid derivatives are widely distributed in plants and are important biologically active constituents of the human diet (*7*, *8*). However, their bioavailability and activity may vary widely depending on their substituents (e.g., glycoside moieties of flavonoids) (*9*). Furthermore, the esterification of glycosides affects the antioxidant properties of flavonoids (*10*). For this reason, it is important to identify and quantify the individual derivatives of phenolic compounds in food products and plant extracts.

Brassica vegetables are a significant source of polyphenols. Several studies have investigated the phenolic composition of members of the Brassicaceae family, including cabbage. Kaempferol and quercetin are the most prevalent flavonoids, glycosylated with one to five sugar moieties and acylated with different hydroxycinnamic acids (*11*–*14*). Some studies have reported the presence of apigenin, luteolin, myricetin, and isorhamnetin in *Brassica* vegetables (*15*–*17*). Furthermore, hydroxycinnamic acids glycosylated or esterified with quinic acid (such as caffeoylquinic acid) were detected (*18*–*20*).

Studies conducted in the 1970s reported the presence of the flavonol glycosides quercetin and isorhamnetin 3-mono- and diglycoside in Chinese cabbage (*21*). The composition of pak choi and Chinese cabbage was reviewed by Herrmann

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(*5*), but few data on phenolic composition were available. The flavonoid apigenin [0.187 mg/g of dry material (dm)] was found in addition to myricetin (0.031 mg/g) in Chinese cabbage, but quercetin, luteolin, and kaempferol were not detected (*16*). Other studies showed the occurrence of kaempferol glycosides and different caffeic acid derivatives in pak choi (*17*). A recent study by Rochfort et al. (*22*) presented a variety of kaempferol, quercetin, and isorhamnetin derivatives in pak choi. The authors determined a total flavonoid aglycone content of different cultivars in the range of 0.7–1.2 mg/g of dm but did not determine the content of hydroxycinnamic acid derivatives.

The aim of this work was to identify the different phenolic compounds in pak choi by means of high-performance liquid chromatography coupled with mass spectroscopy (electrospray ionization source; HPLC–ESI-MS^n) and to elucidate the structure of new compounds by nuclear magnetic resonance spectroscopy (NMR). Furthermore, a quantification of the identified compounds was performed separately in the leaf blade and leaf stem in different pak choi cultivars by HPLC–DAD (diode array detector).

MATERIALS AND METHODS

Chemicals. Acetonitrile (HPLC-grade, Fisher Scientific), Amberlite XAD-4 (Fluka), caffeic acid (Carl Roth GmbH), chlorogenic acid (Sigma), *p*-coumaric acid (Sigma), ferulic acid (Carl Roth GmbH), formic acid (Carl Roth GmbH), isorhamnetin (Sigma), kaempferol (Sigma), methanol (HPLC-grade, Fisher Scientific), oxalic acid dihydrate (Carl Roth GmbH), *m*-phosphoric acid (Fluka), sinapic acid (Carl Roth GmbH), and trifluoroacetic acid (Carl Roth GmbH) were utilized.

Plant Material and Sampling. Eleven varieties of pak choi (*B. campestris* L. ssp. *chinensis* var. *communis*) were cultivated in triplicate in a greenhouse in Northern Germany. Greenhouse temperatures were 25 °C during the day and 20 °C at night. The maximum mean light intensity was 250 μmol m^{-2} s^{-1} measured at noon. Eight-week-old plants were harvested, separated into leaf blades and leaf stems, frozen in liquid nitrogen, and lyophilized. The lyophilized material was powdered, mixed, and stored in the dark until further analysis.

Extraction. Lyophilized plant material (ca. 0.3 g) was mixed with 4 mL of aqueous methanol (50%, v/v) containing 1% *m*-phosphoric acid and 0.5% oxalic acid dihydrate, treated with ultrasonication for 1 min, and centrifuged. The supernatant was collected, and the extraction procedure was repeated three times with 2 mL of solvent. The collected supernatants were made up to 10 mL, filtered, and stored at −20 °C until further analysis.

HPLC Analysis. The HPLC analyses were carried out on an HP1100 HPLC system (Agilent Technology, Waldbronn, Germany) equipped with a diode array detector. Separation was carried out on a 250 mm × 4 mm (inside diameter), 5 μm, RP-18 Nucleodur column with an 8 mm × 4 mm Nucleodur guard column at 20 °C. Eluent A consisted of 0.15% trifluoroacetic acid in water and eluent B of 100% acetonitrile at a flow rate of 0.9 mL/min. Gradient elution started with 10% B for 9 min, reaching 12.5% B after 13 min and 14% B at 19 min. It remained at 14% B until 35 min, reaching 26% B after 60 min, 70% B after 75 min, and 10% B after 77 min up until 80 min. Compounds were detected and quantified by UV absorption at 330 nm. The injection volume was set to 25 μL. Kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside, which was obtained by the isolation procedure (99% pure) described below, was used as a reference compound for quantification (detection limit, 0.002 mg/mL). The quantification of hydroxycinnamic acids was carried out relative to the external standard sinapic acid for the determination of the total and individual contents and relative to ferulic acid, *p*-coumaric acid, caffeic acid, and chlorogenic acid for the determination of the individual contents (detection limit for each standard, 0.001 mg/mL). Further calculations were performed utilizing the molecular weights of the identified compounds [molecular weight factor (mwf) = *M*(identified compound)/*M*(external standard)].

HPLC–DAD–ESI-MS^n. For the qualitative analysis of the polyphenols, the HPLC system was coupled to an Agilent 1100 Series LC/MSD Trap with electrospray ionization. The HPLC conditions were the same as described above except for eluent A, which contained 0.1% formic acid instead of trifluoroacetic acid. The nebulizer pressure was 60 psi and the nitrogen flow rate 10 L/min at a drying temperature of 350 °C. Mass scans were performed from *m*/*z* 50 to 2000 in the negative ionization mode. Helium was used as the collision gas for the fragmentation of the isolated compounds in the ion trap. The detection conditions were as follows: capillary voltage, 3500 V; skimmer voltage, –40 V; cap exit voltage, –158.5 V; Oct1DC, –12 V; Oct2DC, –2.45 V; trap drive level, 45.0; OctRF, 150 Vpp; Lens1, 5.0 V; Lens2, 60 V. MS^n experiments were carried out via an isolation and fragmentation procedure of detected ions.

Hydrolysis Reactions. Alkaline hydrolysis was carried out by the addition of an equal volume of 2 M NaOH to an extract. The solution was stirred overnight, acidified with concentrated HCl (to obtain a pH of <2), and analyzed by HPLC–ESI-MS^n as described above. Acidic hydrolysis was carried out by adding 5 drops of concentrated HCl to the extract and stirring the mixture at 90 °C for 6 h. The extract was directly analyzed by HPLC–MS^n as described above.

Isolation of Polyphenols. Two hundred grams of freeze-dried pak choi (cv. Hangzhou You Dong Er) was mixed with 2 L of distilled water, followed by Ultra-Turrax treatment for 5 min and ultrasonication for 10 min. The mixture was filtered, and the extraction step was repeated three times with 1 L of distilled water each time. The filtrates were collected; 200 g of Amberlite XAD-4 was added, and the mixture was stirred overnight. Afterward, the mixture was filtered through a Buchner funnel, and the Amberlite XAD-4 was washed three times with 100 mL of distilled water. The Amberlite residue was eluted with 2 L of 100% methanol and filtered again through a Buchner funnel. The solvent of the collected filtrate was removed by vacuum distillation, and the residue was stored at −20 °C until further analysis.

The residue was dissolved in a small amount of distilled water, filtered, and prefractionated directly by preparative HPLC. This was performed on a 250 mm × 21 mm (inside diameter), 5 μm, RP-18 Nucleodur column at a flow rate of 20 mL/min and gradient elution. Eluent A consisted of 100% water and eluent B of 100% methanol. The gradient was as follows: 20% B at 0 min, 30% B at 10 min, 60% B at 25 min, and 100% B at 30 min. Detection was carried out by UV absorption at 230 nm for the determination of levels of interfering compounds and 330 nm for the detection of polyphenols by DAD. The injection volume was 1800 μL. The eluted mobile phase was collected in 13 fractions. The purity of the fractions was verified by analytical HPLC as described above. Two fractions were further purified. Fraction 1 (t_R = 2–4.5 min) and fraction 3 (t_R = 11–12 min) were lyophilized and separated again by preparative HPLC to obtain two isolated compounds: kaempferol-3-*O*-hydroxyferuloylsophoroside-7-*O*-glucoside (compound **8**, yellow solid) and sinapoylmalate (compound **22**, not completely purified). UV data, MS^n data, and ^{1}H and ^{13}C data are listed in **Tables 1**, **2**, and **3** and shown in **Figure 3**.

NMR Spectroscopy. The isolated compounds were dissolved in MeOH-d_4, and ^{13}C and ^{1}H, COSY, HSQC, and HMBC spectra were recorded on an Avance 600 MHz spectrometer with CryoProbe (Bruker BioSpin). The reference compound TMS was used as internal standard for the determination of chemical shifts.

RESULTS AND DISCUSSION

The composition and content of phenolic compounds were investigated in freeze-dried plant material from 11 pak choi cultivars separately for the leaf blade and for the leaf stem.

Identification of Aglycones by Hydrolysis. Hydrolysis reactions were carried out to identify the major aglycones of flavonoids and hydroxycinnamic acids in pak choi. Kaempferol was the major aglycone in pak choi to appear after acidic hydrolysis, with a retention time of 70.5 min. The UV spectrum and the retention time were identical to those of the reference compound kaempferol. In addition, small amounts of the flavonoid isorhamnetin were detected with a retention time of

70.8 min, identical to that of the reference compound isorhamnetin. However, quercetin was not detected in pak choi leaves, although another study has reported minor amounts in specimens of this cultivar grown under greenhouse conditions (*22*). The discrepancy in findings could be explained by different growing and climate conditions in greenhouses (e.g., light intensity). After an alkaline hydrolysis reaction, ferulic acid (t_R = 39.5 min) and sinapic acid (t_R = 41.2 min) were detected by HPLC analysis, and retention times were identical to those exhibited by the reference compounds.

Identification of Flavonoids by HPLC–ESI-MS^n. The negative *m*/*z* values $[M - H]^-$ of the detected molecular masses and main fragments MS^n of the identified compounds and their occurrence in the blade and stem are listed in **Table 1**. Panels **A** and **B** of **Figure 1** show two chromatograms of the Shanghai Qing pak choi cultivar and exemplify the qualitative differences between the leaf blade and stem. Twenty-eight compounds were tentatively identified as phenolic structures in pak choi by HPLC–ESI-MS^n. The major flavonoid kaempferol was found as mono-, di-, and triglycosides acylated with different hydroxycinnamic acids. The major hydroxycinnamic acid derivatives were present as esters of quinic acid, malic acid, and glycosides (**Table 1**).

Compounds **8**, **10**, **12**, **14**, and **15** in the leaf blade are monoacylated kaempferoltriglycosides. Each compound showed the loss of 162 amu (atomic mass unit) in the first fragmentation step MS^2, which is the mass of one hexose moiety, followed by one hydroxycinnamic acid moiety by MS^3. Finally, a diglycoside moiety (2 × 162 amu = 324 amu) was split off by MS^4. The fragmentation pattern is in accordance with literature data showing that in *Brassica* species, sugar moieties like glucose are predominantly bound in positions 7 and 3 of the aglycone (*12–14, 20, 22*). During fragmentation, the loss of the sugar moiety from position 7 is more favored than loss from position 3 (*11, 23*).

The detected flavonoid triglycosides differed particularly in their acyl moiety. Losses of 162 amu (caffeic acid) for compound **10**, 206 amu (sinapic acid) for compound **12**, 176 amu (ferulic acid) for compound **14**, and 146 amu (coumaric acid) for compound **15** were seen. The loss of 192 amu (compound **8**) has not been assigned to a structural feature. The same fragmentation pattern seen for compound **8** (**Table 1**) has been described for a flavonoid derivative in cauliflower by ESI-MS^n, but the acyl moiety could not be ascribed to a defined structure (*11*). Another study determined a compound with a molecular weight of 1126 and the fragments MS^n at *m*/*z* 963, 771, and 609 in turnip tops. The authors identified the detected compound as kaempferol-3-methoxycaffeoylsophotrose-7-glucoside (*24*). Due to the difference in mass between the fragments MS^2 at *m*/*z* 963 and MS^3 at *m*/*z* 771 (the loss of 192 amu), they proposed that the acyl moiety is methoxycaffeic acid (also called hydroxyferulic acid). In addition, Rochfort et al. (*22*) also tentatively suggested that the loss of 192 amu corresponds to a dihydroxymethoxycinnamic acid. Because identification by ESI-MS^n was clearly not possible, compound **8** was chosen for NMR structural elucidation.

In addition, flavonoid mono- and diglycoside derivatives of kaempferol and isorhamnetin were detected in the leaf blade (compounds **13**, **16**, **20**, **23**, and **24**). Compound **20** showed the same fragmentation pattern by MS^2 (major fragment at *m*/*z* 285) compared to the fragmentation pattern MS^4 of compounds **5**, **8**, **10**, **12**, **14**, and **15**. The fragmentation step MS^n (609) to *m*/*z* 285 indicates the loss of a diglycoside moiety of an aglycone. The fragmentation pattern of compound **13** differed, showing a

Table 1. Retention Times of the 28 Detected Phenolic Compounds and Their Negative *m*/*z* Values ($[M - H]^-$) of the Molecular Masses and Main Fragments (MS^n) in Extract of Pak Choi Separated in Leaf Blade and Leaf Stem

			m/*z* of the main fragments by ESI-MS^n [relative intensity (%)]				occurrence in leaf[b]	
	t_R (min)	*m*/*z* $[M - H]^-$	MS^2 $[M - H]^-$	MS^3 $[MS^2$ (100%)]	MS^4 $[MS^3$ (100%)]	structure assignment[a]	blade	stem
Flavonoid Derivatives								
5	15.1	771	610 (12), 609 (100)	489 (6), 447 (11), 429 (75), 327 (11), 285 (100), 284 (71), 255 (24)	285 (100), 284 (65), 257 (39), 256 (45), 255 (68), 151 (62)	kaempferol-3-*O*-diglucoside-7-*O*-glucoside	x	–
8	17.2	963	801 (100), 609 (4)	610 (16), 609 (100), 591 (2), 429 (4), 285 (5)	489 (6), 447 (7), 429 (69), 327 (5), 309 (6), 286 (9), 285 (100), 284 (65), 257 (7), 256 (3), 255 (19)	kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside	x	–
10	19.0	933	771 (100), 609 (3)	610 (16), 609 (100), 591 (2), 429 (3), 285 (5)	489 (6), 447 (11), 429 (72), 327 (8), 285 (100), 284 (63), 257 (9), 256 (4), 255 (19)	kaempferol-3-*O*-caffeoyldiglucoside-7-*O*-glucoside	x	–
12	22.1	977	815 (100), 609 (3)	623 (78), 610 (12), 609 (100), 591 (34), 429 (4), 284 (4), 255 (2)	489 (6), 447 (12), 429 (95), 285 (100), 284 (68), 257 (14), 256 (4), 255 (24)	kaempferol-3-*O*-sinapoyldiglucoside-7-*O*-glucoside	x	–
13	23.9	609	488 (15), 447 (100), 285 (15)	447 (80), 327 (19), 285 (56), 284 (100), 255 (17)	284 (7), 257 (2), 256 (25), 255 (100), 227 (13)	kaempferol-3-*O*-glucoside-7-*O*-glucoside	x	–
14	23.9	947	786 (6), 785 (100), 609 (2), 447 (4)	623 (63), 610 (21), 609 (100), 591 (45), 285 (13), 284 (15)	489 (7), 447 (17), 429 (92), 309 (11), 285 (100), 284 (84), 257 (24), 255 (15)	kaempferol-3-*O*-feruloyldiglucoside-7-*O*-glucoside	x	–

Table 1. Continued

	t_R (min)	m/z [M – H]$^-$	m/z of the main fragments by ESI-MS^n [relative intensity (%)]			structure assignment[a]	occurrence in leaf[b]	
			MS^2 [M – H]$^-$	MS^3 [MS^2 (100%)]	MS^4 [MS^3 (100%)]		blade	stem
15	25.8	917	755 (100), 477 (1)	609 (100), 591 (27), 429 (5), 285 (6), 255 (4)	489 (17), 447 (12), 429 (78), 327 (12), 285 (100), 284 (88), 257 (11), 256 (3), 255 (26)	kaempferol-3-*O*-coumaroyldiglucoside-7-*O*-glucoside	x	–
16	25.9	639	519 (13), 477 (100), 315 (8)	357 (16), 315 (58), 314 (100), 285 (12)	314 (80), 300 (30), 286 (65), 285 (100), 271 (81), 257 (35), 255 (13), 243 (20)	isorhamnetin-3-*O*-glycoside-7-*O*-glycoside	x	–
20	46.8	609	489 (4), 447 (10), 429 (65), 285 (100), 284 (71), 255 (24)	285 (100), 284 (58), 257 (56), 256 (6), 255 (35), 151 (73)		kaempferoldiglucoside	(x)	–
23	56.8	447	327 (8), 287 (6), 285 (100), 284 (4), 255 (3)	285 (100), 284 (45), 257 (24), 256 (35), 255 (84), 151 (22)		kaempferolglucoside	(x)	–
24	57.6	477	357 (19), 315 (50), 314 (100), 299 (11), 285 (13)	300 (20), 299 (11), 285 (98), 271 (100), 257 (12), 243 (26)		isorhamnetinglycoside	(x)	–
Hydroxycinnamic Acid Derivatives								
esters of quinic acid and glycoside								
1	8.5	353	257 (5), 191 (100), 179 (34), 135 (5)	173 (61), 127 (100), 111 (34), 109 (34), 93 (79)		monocaffeoylquinic acid (isomer 1)	x	(x)
2	9.5	341	179 (100), 161 (38), 135 (12)	135 (100), 119 (1)		caffeoylglycoside	(x)	–
3	13.1	337	191 (5), 173 (4), 163 (100), 119 (3)	119 (100)		coumaroylquinic acid (isomer 1)	x	(x)
4	14.0	337	191 (5), 173 (3), 163 (100), 119 (5)	119 (100)		coumaroylquinic acid (isomer 2)	x	(x)
6	16.0	367	194 (4), 193 (100), 173 (3), 134 (10)	149 (26), 134 (100), 117 (3)		feruloylquinic acid	(x)	–
7	16.0	353	191 (13), 179 (46), 173 (100), 135 (7)	111 (49), 93 (100), 83 (14), 71 (13)		monocaffeoylquinic acid (isomer 2)	(x)	–
9	18.5	355	311 (20), 193 (100), 173 (3)	178 (42), 149 (95), 134 (100)		feruloylglycoside	(x)	–
11	20.0	385	265 (8), 247 (49), 223 (100), 205 (39), 164 (5)	208 (20), 179 (16), 164 (100), 149 (6)		sinapoylglycoside	(x)	(x)
25	59.8	753	530 (5), 529 (100), 289 (5)	289 (24), 223 (100), 205 (93), 190 (34), 164 (14)	208 (15), 179 (80), 164 (100)	disinapoyldiglycoside	(x)	(x)
26	61.3	723	529 (16), 499 (100), 289 (2), 259 (4)	259 (48), 235 (11), 193 (100), 175 (71)	149 (81), 134 (100)	sinapoylferuloyldiglycoside	(x)	–
27	65.2	959	806 (12), 753 (3), 735 (100), 529 (8), 511 (7), 427 (3)	717 (15), 529 (100), 511 (64), 289 (18), 223 (14)	307 (39), 247 (53), 223 (100), 203 (59)	trisinapoyldiglycoside	(x)	(x)
28	65.9	929	735 (5), 705 (100), 529 (3), 511 (8), 499 (6)	529 (78), 499 (100), 511 (49), 481 (46), 469 (28), 259 (23), 223 (22)	427 (100), 221 (48), 193 (92)	disinapoylferuloyldiglycoside	(x)	–
esters of malic acid								
17	27.7	295	179 (100), 133 (79)	135 (100)		caffeoylmalate	x	–
18	29.9	325	209 (100), 133 (39)	194 (100), 165 (59), 150 (34)		hydroxyferuloylmalate	x	–
19	43.0	279	163 (100), 133 (19)	119 (100)		coumaroylmalate	(x)	–
21	47.9	309	290 (2), 193 (100), 133 (2)	178 (6), 149 (19), 133 (100)		feruloylmalate	x	(x)
22	48.8	339	320 (2), 223 (100)	208 (96), 179 (46), 164 (100), 149 (10)		sinapoylmalate	x	x

[a] The diglucoside moiety of compound **8** was clearly identified as a sophoroside moiety by NMR (**Figure 2** and **Table 2**); the diglucoside moieties of compounds **5**, **10**, **12**, **14**, **15**, and **20** can be tentatively identified as sophoroside moieties by the fragmentation pattern (ESI-MS^n) compared to that of compound **8** and literature data (*22*). [b] x, detected in each cultivar; (x), low content, not detected in all cultivars; –, not detected.

Table 2. NMR Data of Kaempferol-3-*O*-hydroxyferuloylsophoroside-7-*O*-glucoside[a] (MeOH-d_4)

carbon[b]	^{13}C shift[c]	proton[b]	^{1}H shift[c,d]
aglycone		aglycone	
2	158.1		
3	134.7		
4	179.2		
5	162.0		
6	100.8	6	6.43 d (2.2)
7	164.2		
8	95.2	8	6.47 d (2.2)
9	157.5		
10	107.6		
1′	122.6		
2′	132.3	2′	7.94 d (8.9)
3′	116.8	3′	6.94 d (8.9)
4′	162.1		
5′	116.8	5′	6.94 d (8.9)
6′	132.3	6′	7.94 d (8.9)
sugar at C-3		sugar at C-3	
1″	98.6	1″	5.26 d (7.9)
2″	82.0	2″	3.58 dd (8.3, 10.2)
3″	75.8	3″	3.81 m
4″	71.5	4″	3.38 m
5″	78.3	5″	3.31 m
6″	62.4	6a″	3.99 m
		6b″	3.76 m
1‴	97.1	1‴	6.17 d (8.2)
2‴	74.9	2‴	4.94 m
3‴	77.6	3‴	3.64 m
4‴	71.3	4‴	3.46 dd (9.3, 9.5)
5‴	77.9	5‴	3.56 m
6‴	62.2	6a‴	3.97 m
		6b‴	3.81 m
sugar at C-7		sugar at C-7	
1⁗	101.3	1⁗	5.16 d (7.7)
2⁗	74.8	2⁗	3.55 m
3⁗	75.1	3⁗	3.87 m
4⁗	71.1	4⁗	3.55 m
5⁗	78.1	5⁗	3.63 m
6⁗	62.2	6a⁗	3.81 m
		6b⁗	3.55 m
acyl		acyl	
1	125.9		
2	109.9	2	6.24 d (1.8)
3	149.2		
4	138.1		
5	146.3		
6	103.6	6	6.23 d (1.8)
MeO-	56.2	**Me**O-	3.69 s
7 (C**H**=CHCOO)	146.8	7 [C**H**=CHCOO]	7.29 d (15.8)
8 (CH=C**H**COO)	115.4	8 [CH=C**H**COO]	6.07 d (15.8)
9 (CH=CH**C**OO)	168.6		

[a] UV, λ_{max} = 268, 330 nm. [b] For numbering, see **Figure 2**. [c] Chemical shifts in parts per million. [d] Coupling constants (*J*) in hertz are given in parentheses.

major fragment mass MS^2 (609) at *m*/*z* 447 and MS^3 (609 → 447) at *m*/*z* 285. This indicated two separate losses of glycoside moieties (2 × 162 amu) and led to the assumption that the structural formula is kaempferol-3-*O*-glucoside-7-*O*-glucoside. Similarly, compound **16** ($[M - H]^-$ at *m*/*z* 639), with the major fragments MS^2 at *m*/*z* 477 and MS^3 (639 → 477) at *m*/*z* 315 (58% relative intensity), exhibited two separate losses of a glycoside moiety and was identified as isorhamnetin-3-*O*-glycoside-7-*O*-glycoside. The positions of the monoglycoside moiety of compounds **23** and **24** and the diglycoside moiety of compound **20** could not be clearly identified by ESI-MS^n. Presumably, the glycoside moieties are bound at position 3 of the aglycone, according to other studies of *Brassica* vegetables (*11*, *12*, *22*).

Furthermore, fragments MS^n (609) at *m*/*z* 489 and 429 were observed for the diglycoside moiety of compounds **5**, **8**, **10**, **12**, **14**, **15**, and **20** and confirm the connection of a diglucoside moiety of the aglycone. A detailed description of the fragmentation pattern of the sugar moieties was given by Rochfort et al. (*22*). They showed that the losses of 180 and 120 amu are significant for diglucosides like sophorosides (1 → 2 linkages of two glucose molecules). Additionally, in this study, the monoglycoside moieties represent the loss of 120 amu [e.g., compound **16**, MS^2 (639) at *m*/*z* 519 (3% relative intensity); and compound **23**, MS^2 (447) at *m*/*z* 327 (8% relative intensity)].

Rochfort et al. (*22*) detected several kaempferoldiglucosides esterified with one acyl moiety in pak choi. However, these compounds were not detected in our study.

Flavonoids with four or more glycoside moieties as presented for white cabbages by Nielsen et al. (*14*) or cauliflower by Llorach et al. (*11*) have not been identified for pak choi.

Each presumed kaempferol derivative showed significant fragment masses of the aglycone MS^n at *m*/*z* 285. The fragment masses MS^{n+1} at *m*/*z* 257, 256, and 255 are generated by the loss of the CO of the fragment masses MS^{n+1} at *m*/*z* 285, 284, and 283 (*m*/*z* 283 was not detected in this study) of the aglycone (*25*, *26*). MS^{n+1} at *m*/*z* 255 can also be generated by the loss of CH_2O of the fragment mass at *m*/*z* 285 (*27*). The fragment masses MS^n (314) at *m*/*z* 285, 257, and 255 were also detected for the isorhamnetin derivatives (compounds **16** and **24**). The fragment mass MS^{n+1} at *m*/*z* 151 shown for compounds **20** and **23** by MS^3 and compound **5** by MS^4 is characteristic for ring A of the flavonol kaempferol depending on the hydroxyl groups at positions 5 and 7. Other studies have shown that a fragment with the mass MS^{n+1} at *m*/*z* 151 is typical for several flavonoids (*26*, *28*). Kaempferol possesses the same *m*/*z* value $[M - H]^-$ for the molecule as the flavone luteolin in particular. Therefore, the identification by acidic hydrolysis reactions was necessary to provide clear evidence that compounds **5**, **8**, **10**, **12–15**, **20**, and **23** are kaempferol derivatives.

Structural Determination of Compound 8 by NMR. ^{1}H and ^{13}C NMR spectroscopy, including COSY, HSQC, and HMBC, resulted in a complete structural determination of compound **8** and confirmed the proposed acyl moiety as being hydroxyferulic acid as well as the assignment of the positions of the three glycoside moieties by ESI-MS^n. The complete structure was identified as kaempferol-3-*O*-hydroxyferuloylsophoroside-7-*O*-glucoside (**Figure 2**).

The NMR shifts of the aglycone part, the sugar moieties at positions 3 and 7, and the acyl moiety are listed in **Table 2**. The signals of C-3 and C-7 of kaempferol are shifted downfield, indicating the attachment of sugar substituents. The signal at δ 134.7 indicates a sugar linkage at position C-3. The aglycone standard of kaempferol (no glycoside linkages) shows a chemical shift of δ 137.1 for C-3 (data not shown). C-7 (δ 164.2) is linked with another monoglycoside moiety [C-7 of the kaempferol aglycone standard, δ 165.7 (data not shown)]. The ^{13}C NMR shifts of C-3 and C-7 are in agreement with published data for acylated kaempferol-3-*O*-sophoroside-7-*O*-glucosides, measured in DMSO-d_6 (*13*). The attached sugar substituents can be identified as glucoside and sophoroside moieties by the characteristic ^{1}H and ^{13}C shifts (COSY, HSQC, and HMBC). The shifts are also comparable with literature data for acylated kaempferol-3-*O*-sophoroside-7-*O*-glucosides (measured in DMSO-d_6) (*13*) and confirm the glucoside moieties, which have been proposed as substituents for all kaempferol derivatives on the basis of HPLC–ESI-MS^n (**Table 1**). ^{13}C NMR shifts of the acyl moiety are similar to those of the sinapic acid residue detected for kaempferol-3-*O*-sinapoylsophoroside-7-*O*-glucoside by Nielsen et al. (*13*) in cabbage leaves. However, C-3 (δ 149.2) and C-5

Table 3. NMR Data of Sinapoylmalate[a] (MeOH-d_4)

carbon[b]	^{13}C shift[c]	proton[b]	^{1}H shift[c,d]
hydroxycinnamic acid moiety		hydroxycinnamic acid moiety	
1	127.1		
2	106.8	2	6.93 s
3	149.5		
4	139.3		
5	149.5		
6	106.8	6	6.93 s
MeO (C-3)	56.9	**Me**O (C-3)	3.92 s
MeO (C-5)	56.9	**Me**O (C-5)	3.92 s
7 (**C**H=CHCOO)	146.3	7 (C**H**=CHCOO)	7.65 d (15.9)
8 (CH=**C**HCOO)	117.0	8 (CH=C**H**COO)	6.45 d (15.9)
9 (CH=CH**C**OO)	169.1		
organic acid moiety		organic acid moiety	
1′	178.4		
2′	75.3	2′	5.39 dd (3.0, 10.6)
3′	41.5	3a′	2.86 dd (3.0, 15.7)
		3b′	2.75 dd (10.6, 15.7)
4′	178.0		

[a] For UV data, see **Figure 3**. [b] For numbering, see **Figure 3**. [c] Chemical shifts in parts per million. [d] Coupling constants (*J*) in hertz are given in parentheses.

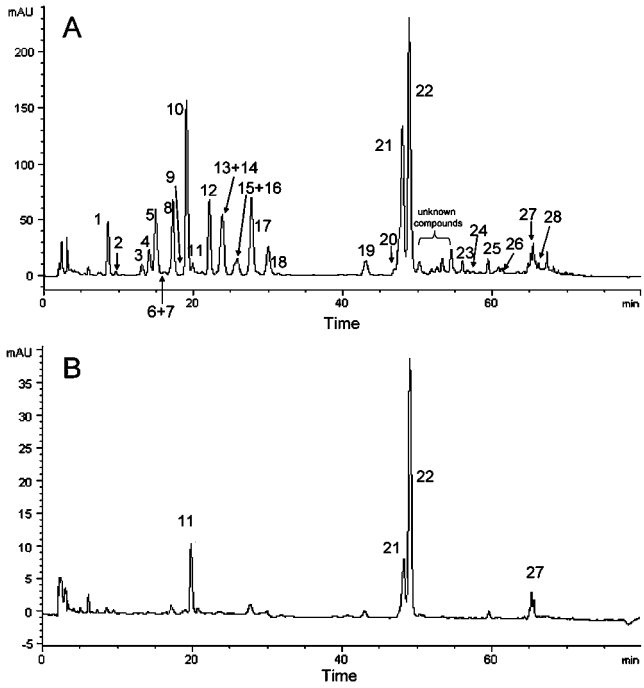

Figure 1. HPLC–DAD chromatograms of the aqueous methanolic extract of pak choi cv. Shanghai Qing: (**A**) leaf blade and (**B**) leaf stem, monitored at 330 nm.

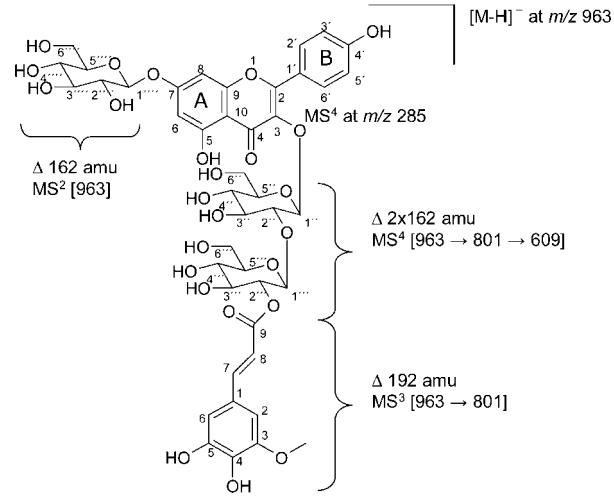

Figure 2. Structure of compound **8** as elucidated by NMR and its fragmentation by ESI-MS^n.

(δ 146.3) of the acyl moiety differ compared to the detected shifts by Nielsen et al. (*13*) for the sinapic residue (δ 147.8 for both C-3 and C-5 in DMSO-d_6). The detailed NMR analysis (^{1}H, ^{13}C, COSY, HSQC, and HMBC) indicates that the acyl moiety is hydroxyferulic acid. A significant correlation between C-3 and the protons of the methoxy group at C-3 was observed by HMBC. The shifts of the acyl moiety are also in accordance with the literature data shown for the hydroxyferuloyl moiety of the compound 5-hydroxyferuloylmalate (*29*). To our knowledge, the structural determination for compound **8** by means of NMR (including the hydroxyferulic acid as the acyl moiety of flavonoids) is described here for the first time.

Identification of Hydroxycinnamic Acids by HPLC–MS^n. Different derivatives of hydroxycinnamic acids (like hydroxyferulic acid, ferulic acid, caffeic acid, coumaric acid, and sinapic acid) were detected in the leaf blade, and to a lesser extent in the leaf stem, of different pak choi cultivars (**Table 1**).

Hydroxycinnamic acids, such as esters of quinic acid, were identified in pak choi leaves. The detected mass $[M - H]^-$ at *m*/*z* 337 of two compounds, **3** and **4**, showed a major fragment mass MS^2 at *m*/*z* 163, which represented the fragment mass of coumaric acid of two different coumaroylquinic acid isomers. The detected monocaffeoylquinic acid (compound **1**) with $[M - H]^-$ at *m*/*z* 353 showed the major fragment masses MS^2 at *m*/*z* 191 (for quinic acid, such as compounds **3** and **4**) and *m*/*z* 179 (for caffeic acid; relative intensity of 34%) as well as the fragment mass MS^3 (353 → 191) at *m*/*z* 173 (61% intensity) for the fragment trihydroxycyclohexanecarboxylic acid, which was previously reported by Poon (*30*) and Fang (*31*). Compound **7** was identified as another monocaffeoylquinic acid isomer, and the fragmentation procedure showed the major fragment mass MS^2 at *m*/*z* 173. Additionally, the mass $[M - H]^-$ at *m*/*z* 367 of compound **6** represented the compound feruloylquinic acid with the major fragment mass MS^2 at *m*/*z* 193 (for ferulic acid); a fragment mass MS^2 at *m*/*z* 191 was not detectable.

Furthermore, this analysis showed a number of hydroxycinnamic acid esters with different glycoside moieties. Three hydroxycinnamic acid derivatives esterified with one glycoside moiety were identified. These compounds are monoglycosides

of caffeic acid (compound **2**, $[M - H]^-$ at *m/z* 341), ferulic acid (compound **9**, $[M - H]^-$ at *m/z* 355), and sinapic acid (compound **11**, $[M - H]^-$ at *m/z* 385). Each compound exhibited the loss of 162 amu by MS^2 and the major fragment masses at *m/z* 179, 193, and 223 for the hydroxycinnamic acid aglycones. Other identified hydroxycinnamic acid derivatives that carry more than one glycoside moiety are disinapoyldiglycoside (compound **25**) and sinapoylferuloyldiglycoside (compound **26**), which showed the negative molecular masses $[M - H]^-$ at *m/z* 753 (compound **25**) and *m/z* 723 (compound **26**). These are in accordance with compounds detected in other *Brassica* species, like turnip tops (*24*) and tronchuda cabbage (*20*). The fragmentation step MS^2 (753) with the fragment mass at *m/z* 529 showed the loss of 224 amu for compound **25**. Additionally, the fragmentation step MS^2 (723) of compound **26** with the fragment masses at *m/z* 499 and 529 (lower relative intensity) showed the loss of 224 amu and, to a lesser extent, of 194 amu. This represents the loss of one hydroxycinnamic acid moiety like sinapic acid (224 amu, compound **25**) as well as sinapic acid and ferulic acid (224 and 193 amu, respectively, compound **26**). Thus, it was observed that the loss of one sinapic acid moiety of compound **28** is more favored by ESI-MS^2 than the loss of one ferulic acid moiety. Furthermore, the loss of 306 amu represented the loss of two glycoside moieties, resulting in the fragment masses MS^3 (753 → 529) at *m/z* 223 (sinapic acid) for compound **25** and MS^3 (723 → 499) at *m/z* 193 (ferulic acid) for compound **26**. In the literature, the diglycoside moiety is often described as a gentiobiose unit (1 → 6 linkage of two glucoside molecules) (*18*, *20*, *32*).

Compounds **27** and **28**, with masses $[M - H]^-$ at *m/z* 959 and 929, were identified as isomers of trisinapoyldiglycoside and disinapoylferuloyldiglycoside according to Ferreres et al. (*20*). The identified major fragment masses MS^2 at *m/z* 735 (compound **27**) and *m/z* 705 (compound **28**) are comparable with the detected fragment masses in the literature (*20*) and show the loss of 224 amu (sinapic acid moiety) for both compounds. The fragment masses MS^3 (959 → 735) at *m/z* 529 (compound **27**) and MS^3 (929 → 705) at *m/z* 499 (compound **28**) indicate the further loss of 206 amu as a sinapic acid moiety for both compounds. The fragmentation by MS^4 of compound **27** (*m/z* at 223) and compound **28** [*m/z* 193 (92% relative intensity)] was in accordance with that of compounds **25** and **26**.

Malic acid derivatives of hydroxycinnamic acids (compounds **17–19**, **21**, and **22**) and their proposed structures (fragmentation pattern by MS^2) and UV data are listed in **Table 1** and shown in **Figure 3**. The mass $[M - H]^-$ at *m/z* 325 and the resulting fragment mass MS^2 at *m/z* 209 (compound **18**) were detected and identified as an ester of malic acid with hydroxyferulic acid. Four more compounds with the masses $[M - H]^-$ at *m/z* 295, 279, 309, and 339 were detected and identified as different esters of malic acid with caffeic acid, coumaric acid, ferulic acid, and sinapic acid, respectively. The loss of 116 amu by MS^2 is characteristic for the five malic acid derivatives, which is seen in **Figure 3**. The fragment mass MS^2 at *m/z* 133 was significant for each malic acid derivative and showed the fragment of malic acid. The fragmentation pattern corresponds to data in the literature (*29*). To our knowledge, these five different hydroyxcinnamic acid derivatives of malic acid are described in cabbages for the first time.

Information about the presence of malic acid moieties of hydroxycinnamic acids is scarce in the literature. It has been reported that caffeoylmalate occurs in lettuce (*33*) as well as in herbs like white horehound (*Marrubium vulgare*) (*34*). In radishes, which also belong to the Brassicaceae family, different

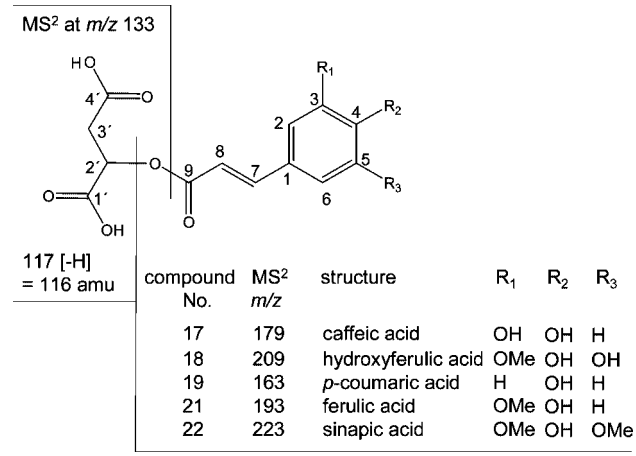

Figure 3. Fragmentation pattern by ESI-MS^n of five identified hydroxycinnamoylmalates in pak choi leaves. UV data of the identified malate derivatives in pak choi leaves: caffeoylmalate (**17**), 305sh and 330max; hydroxyferuloylmalate (**18**), 328max; coumaroylmalate (**19**), 312max; feruloylmalate (**21**), 300sh and 328max; and sinapoylmalate (**22**), 330max.

malic acid esters of caffeic acid, *p*-coumaric acid, sinapic acid, and ferulic acid have previously been found (*35*, *36*). It is also known that sinapoylmalate occurs in *Arabidopsis thaliana*. Goujon et al. (*37*) detected this compound by MS spectroscopy and recorded its fragmentation pattern as shown in **Figure 3**. Furthermore, the authors identified compounds with the masses $[M - H]^-$ at *m/z* 325 and 371 and the fragment mass MS^2 at *m/z* 209 (both compounds) as 5-hydroxyferuloylmalate and 5-hydroxyferuloylglycoside. Liang et al. (*29*) identified five malate derivatives in turnip leaves (*Brassica rapa*) by NMR spectroscopy with the assistance of HPLC–MS, including hydroxyferuloylmalate as a new compound. Rochfort et al. (*22*) also detected two major compounds in pak choi with the molecular masses $[M - H]^-$ at *m/z* 309 and 339 but were unable to identify them.

Structural Determination by NMR of Compound 22. Sinapoylmalate (compound **22**) was isolated with contamination that could not be separated by preparative HPLC. However, a complete structural determination of the sinapoylmalate of the cabbage was possible. The determination was carried out by ^1H and ^{13}C NMR, including COSY, HSQC, and HMBC spectra, to confirm by this example the structure of malate derivatives of hydroxycinnamic acids, which were identified by ESI-MS^n. The NMR data are presented in **Table 3**. They include typical shifts for the malate moiety and sinapic acid comparable to those documented in the literature on malate derivatives from *Raphanus sativus* (^1H NMR shifts) and *B. rapa* (^1H and ^{13}C NMR shifts of the malate derivative mixture) (*29*, *35*).

The presence of sinapoylmalate in pak choi can be seen as an indication of the activity of the enzyme L-malate-*O*-sinapoyltransferase, an enzyme which is involved in the biosynthesis of malic acid esters in plant material and found in different species of the Brassicaceae family like *A. thaliana*, *R. sativus* (radish), and *Brassica napus* (rape) (*38–41*).

Quantification of Polyphenols. Quantitative determination of the identified compounds was carried out in triplicate for 11 different cultivars of pak choi (**Tables 4–7**). All flavonoid and hydroxycinnamic acid derivatives were quantified individually in the plant extracts (**Figure 1A,B**). Flavonoids were detected only in the leaf blade of all cultivars, whereas hydroxycinnamic acids were detected in the leaf blade and, to a smaller extent, in the leaf stem.

Table 4 shows the total contents for flavonoid derivatives in the leaf blade. The contents were expressed as kaempferol-3-

Table 4. Total Contents of Flavonoid Derivatives in the Leaf Blade of 11 Cultivars of Pak Choi

cultivar	flavonoid derivatives (mg/g of dm)[a]	aglycones (mg/g of dm)[b]
Ai Kang Qing	8.32 ± 0.27	2.47 ± 0.08
Lu Xiu	9.56 ± 0.74	2.84 ± 0.22
Suzhou Qing	5.18 ± 1.54	1.54 ± 0.46
Shanghai Qing	8.44 ± 1.15	2.50 ± 0.34
Nanjing Zhong Gan Bai	9.18 ± 1.89	2.72 ± 0.56
Hei You Bai Cai	6.15 ± 0.55	1.82 ± 0.16
Al Jiao Huang	5.04 ± 1.24	1.50 ± 0.37
Si Yue Man	7.23 ± 1.68	2.14 ± 0.50
Si Ji Xiao Bai Cai	7.41 ± 2.32	2.20 ± 0.69
Huang Xin Cai	4.68 ± 0.21	1.39 ± 0.06
Hangzhou You Dong Er	16.67 ± 1.20	4.95 ± 0.36
mean value[c]	7.99 ± 3.34	2.37 ± 0.99

[a] Expressed as kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside equivalents. [b] Calculated on the basis of kaempferol aglycone. [c] Mean value for the contents of all cultivars together.

Table 5. Total Contents of Hydroxycinnamic Acid Derivatives in Leaf Blade and Leaf Stem of 11 Cultivars of Pak Choi

cultivar	blade (mg/g of dm)[a]	stem (mg/g of dm)[a]
Ai Kang Qing	2.98 ± 0.03	0.33 ± 0.02
Lu Xiu	3.72 ± 0.22	0.43 ± 0.09
Suzhou Qing	3.46 ± 0.31	0.40 ± 0.07
Shanghai Qing	3.65 ± 0.40	0.48 ± 0.23
Nanjing Zhong Gan Bai	4.37 ± 0.26	1.02 ± 0.31
Hei You Bai Cai	4.18 ± 0.22	1.07 ± 0.08
Al Jiao Huang	3.98 ± 0.16	0.43 ± 0.07
Si Yue Man	3.49 ± 0.08	0.45 ± 0.04
Si Ji Xiao Bai Cai	3.49 ± 0.43	0.96 ± 0.22
Huang Xin Cai	5.83 ± 0.43	1.36 ± 0.14
Hangzhou You Dong Er	1.48 ± 0.06	0.61 ± 0.18
mean value[b]	3.69 ± 1.04	0.69 ± 0.35

[a] Calibrated by sinapic acid and further calculated by the molecular weight factor (mwf) of the main hydroxycinnamic acid derivative sinapoylmalate (mwf = 340/224). [b] Mean value for the contents of all cultivars together.

O-hydroxyferuloyldiglucoside-7-*O*-glucoside equivalents, because triglucoside derivatives represented approximately 90–95% of the flavonoid derivatives. In addition, the flavonoid contents were calculated on the basis of kaempferol aglycone (**Table 4**), the main flavonoid, so the data of this study can be compared with results in the literature. The concentration of flavonoid derivatives in the leaf blade ranged from 4.68 mg/g of dm (cv. Huang Xin Cai) to 16.67 mg/g of dm (cv. Hangzhou You Dong Er), expressed as kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside equivalents. The results for the total amounts of flavonoids equal to 4.86–17.29 μmol/g of dm are in accordance with the results of Sakakibara et al. (*17*), who reported the total amount of 0.53–1.33 μmol/g of fresh material (approximately 5–13 μmol/g of dm) for kaempferol glycoside derivatives in the whole leaves of pak choi. Rochfort et al. (*22*) detected total contents for the aglycones kaempferol, isorhamnetin, and quercetin in the range of 0.7–1.2 mg/g of dm in pak choi. These results were obtained for aglycones after hydrolysis reactions. Considering that the flavonoid contents were reported for the whole leaves (blade and stem), the contents were in the same range as for pak choi cultivars in our study. It should also be kept in mind that a potential degradation may have been caused by the hydrolysis reaction utilized by Rochfort et al. (*22*).

The total content of hydroxycinnamic acids was expressed as sinapoylmalate (milligrams per gram of dry material), as this compound was found to be the major hydroxycinnamic acid derivative (**Table 5**). The concentration of hydroxycinnamic acid derivatives ranged from 1.48 mg/g of dm (cv. Hangzhou You Dong Er) to 5.83 mg/g of dm (cv. Huang Xin Cai) in leaf blades and from 0.33 mg/g of dm (cv. Ai Kang Qing) to 1.36 mg/g of dm (cv. Huang Xin Cai) in leaf stems. The results for the total hydroxycinnamic acid amount in leaf blade equal to 4.36–17.14 μmol/g of dm are in accordance with the study by Sakakibara et al. (*17*), who found a total content of 0.24–0.89 μmol/g of fresh material [approximately 2–8 μmol/g of dm (whole leaf)] for caffeic acid, chlorogenic acid, and cinnamic acids in pak choi.

A comparison of the flavonoid and hydroxycinnamic acid contents of leaf blades indicates that a high content of flavonoids seems to be associated with a low content of hydroxycinnamic acids and vice versa, particularly the comparison of cv. Hangzhou You Dong Er and cv. Huang Xin Cai (**Tables 4** and **5**).

The quantitative amounts of selected compounds possessing significant concentrations in each cultivar are presented in **Tables 6** and **7**.

The major flavonoid derivatives and their individual contents in the leaf blade are presented in **Table 6**. The concentrations of the individual flavonoids differed strongly among the cultivars. Compound **8**, for example, ranged from 0.48 mg/g of dm (cv. Ai Kang Qing) to 2.26 mg/g of dm (cv. Nanjing Zhong Gan Bai). The concentrations for compounds **15** and **16** were always low except for cv. Hangzhou You Dong Er (2.22 mg/g of dm). The cv. Hangzhou You Dong Er possessed the highest contents for most of the indicated flavonoid derivatives, whereas the cv. Huang Xin Cai exhibited the lowest contents (**Table 6**).

Table 6. Contents[a] (milligrams per gram of dry material) of Flavonoid Derivatives in the Leaf Blade of 11 Cultivars of Pak Choi

cultivar	**5**	**8**	**10**	**12**	**13 + 14**	**15 + 16**
Ai Kang Qing	1.03 ± 0.45	0.48 ± 0.10	2.37 ± 0.30	1.02 ± 0.15	2.06 ± 0.33	0.27 ± 0.07
Lu Xiu	1.24 ± 0.27	1.02 ± 0.41	2.76 ± 0.37	1.11 ± 0.10	2.11 ± 0.53	0.31 ± 0.03
Suzhou Qing	0.39 ± 0.18	0.85 ± 0.33	1.76 ± 0.27	0.54 ± 0.21	0.98 ± 0.36	nq[b]
Shanghai Qing	1.28 ± 0.37	1.22 ± 0.35	2.40 ± 0.50	1.09 ± 0.27	1.24 ± 0.34	0.29 ± 0.13
Nanjing Zhong Gan Bai	1.16 ± 0.48	2.26 ± 0.67	2.09 ± 0.04	1.13 ± 0.11	1.59 ± 0.42	nq
Hei You Bai Cai	0.51 ± 0.23	1.42 ± 0.30	1.76 ± 0.25	0.69 ± 0.27	0.99 ± 0.22	nq
Al Jiao Huang	0.40 ± 0.20	1.09 ± 0.18	1.31 ± 0.23	0.55 ± 0.16	1.21 ± 0.32	nq
Si Yue Man	0.54 ± 0.22	1.06 ± 0.22	2.30 ± 0.44	1.07 ± 0.37	1.50 ± 0.29	nq
Si Ji Xiao Bai Cai	0.82 ± 0.61	1.79 ± 0.37	1.17 ± 0.39	0.62 ± 0.23	1.05 ± 0.42	nq
Huang Xin Cai	0.33 ± 0.13	1.41 ± 0.20	1.08 ± 0.17	0.50 ± 0.07	0.72 ± 0.25	nq
Hangzhou You Dong Er	2.58 ± 0.55	1.34 ± 0.21	2.68 ± 0.09	2.43 ± 0.21	3.35 ± 0.52	2.22 ± 0.19

[a] Calibrated by kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside and further calculated by the mwf of the individual compounds: compound **5**, kaempferol-3-*O*-diglucoside-7-*O*-glucoside, mwf = 772/964; compound **8**, kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside; compound **10**, kaempferol-3-*O*-caffeoyldiglucoside-7-*O*-glucoside, mwf = 934/964; compound **12**, kaempferol-3-*O*-sinapoyldiglucoside-7-*O*-glucoside, mwf = 978/964; compound **13**, kaempferol-3-*O*-glucoside-7-*O*-glucoside, and compound **14**, kaempferol-3-*O*-feruloyldiglucoside-7-*O*-glucoside, mwf = [(610 + 948)/2]/964; compound **15**, kaempferol-3-*O*-coumaroyldiglucoside-7-*O*-glucoside, and compound **16**, isorhamnetin-3-*O*-glycoside-7-*O*-glycoside, mwf = [(918 + 640)/2]/964. [b] Not quantified.

Table 7. Contents (milligrams per gram of dry material) of Hydroxycinnamic Acid Derivatives in the Leaf Blade of 11 Cultivars of Pak Choi (contents for compound **22** in blades and stems)

cultivar	**1**[a]	**3** + **4**[b]	**17**[c]	**18**[d]	**21**[d]	**22**[e] (blade)	**22**[e] (stem)
Ai Kang Qing	0.26 ± 0.08	0.23 ± 0.08	0.30 ± 0.05	nq[f]	1.01 ± 0.03	0.62 ± 0.08	0.21 ± 0.02
Lu Xiu	0.42 ± 0.09	0.41 ± 0.12	0.36 ± 0.11	nq	1.03 ± 0.14	0.90 ± 0.03	0.31 ± 0.07
Suzhou Qing	0.27 ± 0.05	0.25 ± 0.06	0.45 ± 0.05	0.19 ± 0.02	0.92 ± 0.13	0.83 ± 0.05	0.27 ± 0.05
Shanghai Qing	0.42 ± 0.08	0.34 ± 0.10	0.34 ± 0.05	0.16 ± 0.07	0.86 ± 0.07	1.09 ± 0.18	0.32 ± 0.16
Nanjing Zhong Gan Bai	0.36 ± 0.19	0.32 ± 0.13	0.41 ± 0.05	0.37 ± 0.02	1.09 ± 0.27	1.24 ± 0.17	0.75 ± 0.24
Hei You Bai Cai	0.53 ± 0.25	0.59 ± 0.31	0.23 ± 0.05	0.20 ± 0.10	0.88 ± 0.10	1.30 ± 0.24	0.80 ± 0.07
Al Jiao Huang	0.45 ± 0.15	0.44 ± 0.12	0.36 ± 0.05	0.16 ± 0.02	1.13 ± 0.03	0.96 ± 0.18	0.31 ± 0.06
Si Yue Man	0.42 ± 0.12	0.46 ± 0.05	0.36 ± 0.09	nq	0.86 ± 0.06	0.86 ± 0.14	0.29 ± 0.05
Si Ji Xiao Bai Cai	0.64 ± 0.09	0.72 ± 0.26	nq	nq	0.57 ± 0.16	0.76 ± 0.10	0.53 ± 0.25
Huang Xin Cai	0.64 ± 0.15	0.29 ± 0.07	0.32 ± 0.07	0.32 ± 0.12	1.49 ± 0.08	1.88 ± 0.29	1.00 ± 0.06
Hangzhou You Dong Er	nq	0.24 ± 0.03	nq	nq	0.34 ± 0.01	0.47 ± 0.06	0.49 ± 0.19

[a] Expressed as chlorogenic acid equivalents [compound **1**, monocaffeoylquinic acid (isomer 1)]. [b] Calibrated by *p*-coumaric acid and further calculated by the mwf [compound **3**, coumaroylquinic acid (isomer 1), and compound **4**, coumaroylquinic acid (isomer 2), mwf = 338/164]. [c] Calibrated by caffeic acid and further calculated by the mwf (compound **17**, caffeoylmalate, mwf = 296/180). [d] Calibrated by ferulic acid and further calculated by the mwf (compound **18**, hydroxyferuloylmalate, mwf = 326/210; compound **21**, feruloylmalate, mwf = 310/194). [e] Calibrated by sinapic acid and further calculated by the mwf (compound **22**, sinapoylmalate, mwf = 340/224). [f] Not quantified.

However, in some cases, the standard variation within one cultivar was remarkably high (e.g., 0.67 for compound **8**, cv. Nanjing Zhong Gan Bai).

The major hydroxycinnamic acid derivatives, such as quinic acid derivatives (compounds **1**, **3**, and **4**) and malate derivatives (compounds **17**, **18**, **21**, and **22**) and their individual contents in the leaf blade are expressed in terms of their molecular weights (**Table 7**). In addition, the content of sinapoylmalate (compound **22**) in the leaf stem is given. Sinapoylmalate was found to be the major hydroxycinnamic acid and the major polyphenol in the stems of the pak choi cultivars under investigation. Low contents were detected for feruloylmalate (**Figure 1B** and **Table 1**) in stems of all cultivars and were not quantified. Coumaroylmalate (compound **19**) as well as other hydroxycinnamic acid derivatives like glycosides were present only in trace amounts. The contents of hydroxyferuloylmalate and caffeoylmalate were significantly lower than those of feruloylmalate and sinapoylmalate in the leaf blades of the 11 cultivars. The maximum concentrations of the malate derivatives that were found amounted to 0.45 mg/g of dm for caffeoylmalate (compound **17**, cv. Suzhou Qing), 0.37 mg/g of dm for hydroxyferuloylmalate (compound **18**, cv. Nanjing Zhong Gan Bai), 1.49 mg/g of dm for feruloylmalate (compound **21**, cv. Huang Xin Cai), and 1.88 mg/g of dm for sinapoylmalate (compound **22**, cv. Huang Xin Cai). The concentrations of sinapoylmalate in the leaf stem ranged from 0.21 mg/g of dm (cv. Ai Kang Qing) to 1.00 mg/g of dm (cv. Huang Xin Cai). Nielsen et al. (*36*) detected high contents of coumaroylmalate (1.88 mg/g of dm), caffeoylmalate (1.98 mg/g of dm), and feruloylmalate (1.22 mg/g of dm) in leaves of 28-day-old *R. sativus* plants but lower contents for sinapoylmalate (0.15 mg/g of dm). Hydroxyferuloylmalate was not detected, however.

In addition to growing conditions, plant age, and genotype (*42*), the weight ratio of blade to stem influenced the total content of polyphenols in the leaves of the pak choi cultivars. The detected blade/stem ratios for the 11 cultivars based on fresh weight were as follows: 0.48 for cv. Ai Kang Qing, 0.43 for cv. Lu Xiu, 0.44 for cv. Suzhou Qing, 0.54 for cv. Shanghai Qing, 0.50 for cv. Nanjing Zhong Gan Bai, 0.34 for cv. Hei You Bai Cai, 0.49 for cv. Al Jiao Huang, 0.61 for cv. Si Yue Man, 0.62 for cv. Si Ji Xiao Bai Cai, 0.57 for cv. Huang Xin Cai, and 0.23 for cv. Hangzhou You Dong Er. A smaller proportion of stem increases the total quantitative content of polyphenols in total leaves, which is dependent on the cultivar and influences the intake of dietary polyphenols in human nutrition. The Si Yue Man and Si Ji Xiao Bai Cai cultivars possessed the highest blade/stem ratio (0.61–0.62), for example. Furthermore, the growing conditions may affect quantitative as well as qualitative differences, particularly with respect to the occurrence of quercetin derivatives. Slight differences in growing conditions might explain why no quercetin derivatives were found in our study; Rochfort et al. (*22*) meanwhile identified four different quercetin derivatives, such as quercetindi- and -triglycosides under greenhouse conditions. However, in their investigation, the intensity of the applied light was approximately 3-fold [53.8 klx = approximately 750 μmol m^{-2} s^{-1} (mean maximum light level)] greater than that used in our study (approximately 250 μmol m^{-2} s^{-1}), and there is no detailed information about the light quality. It was shown that the biosynthesis of quercetin, particularly quercetin-3-*O*-rutinoside, is induced by UV-B exposition and water deficiency (*43–45*), which could be causes for the observed differences.

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