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A Comparative Study of Flavonoid Compounds, Vitamin C, and Antioxidant Properties of Baby Leaf *Brassicaceae* Species

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A comparative study of antioxidant compounds, flavonoids and vitamin C, and also antioxidant activity was carried out in four species of *Brassicaceae* vegetables used for salads: watercress (*Nasturtium officinale* R. Br.), mizuna [*Brassica rapa* L. subsp. *nipposinica* (L.H. Bailey) Haneltand], wild rocket [*Diplotaxis tenuifolia* (L.) DC.], and salad rocket [*Eruca vesicaria* (L.) Cav.]. The characterization of individual phenolic compounds by HPLC-DAD-MS/MS-ESI in watercress and mizuna completes the polyphenol study previously reported for wild rocket and salad rocket. The qualitative study of flavonoids in watercress leaves showed a characteristic glycosylation pattern with rhamnose at the 7 position. Isorhamnetin 3,7-di-*O*-glucoside was identified in mizuna leaves and may be considered a chemotaxonomical marker in some *B. rapa* subspecies. *Brassicaceae* species showed differences in the quantitative study of flavonoids, and the highest content was detected in watercress leaves. Watercress and wild rocket leaves had the highest content of vitamin C. The antioxidant activity evaluated by different methods (ABTS, DPPH, and FRAP assays) showed a high correlation level with the content of polyphenols and vitamin C. In conclusion, the *Brassicaceae* leaves studied, watercress, mizuna, wild rocket, and salad rocket, presented a large variability in the composition and content of antioxidant compounds. These baby leaf species are good dietary sources of antioxidants with an important variability of bioactive compounds.

KEYWORDS: Wild rocket; salad rocket; watercress; mizuna; phenolics; mass spectrometry; antioxidant activity

INTRODUCTION

In recent years, great importance has been attached to the consumption of fresh-cut vegetables for health reasons. The beneficial effects have been attributed to the antioxidant vitamins such as ascorbic acid, β-carotene, and α-tocopherol present in vegetables (*1*). Moreover, other different compounds such as polyphenols have been studied in relation to their content and antioxidant capacity (*2*). Among the diverse functions of these compounds, the antioxidant properties are very important and depend on the stability of compounds in different systems, as well as number and localization of hydroxyl groups (*3*). However, in plant foods, flavonols are not found free but rather as complex conjugates with sugar residues. These sugar residues may also be linked to hydroxycinnamic residues (*4–6*). Polyphenols and vitamins are present in cruciferous vegetables in large quantities (*7*). The most studied cruciferous vegetables belong to the *Brassica* genera including broccoli, cabbage, kale, etc. However, other new leaf species belonging to the *Brassicaceae* family have been recently introduced as ingredients for salads, such as rocket, watercress, and mizuna.

Rocket includes different species belonging to *Eruca* and *Diplotaxis* genera, with a distinct spicy flavor, due to the hydrolytic products of some glucosinolates (*8, 9*). Previous works have reported the characterization of the polyphenol profile of *D. tenuifolia* and *E. vesicaria*, with quercetin and kaempferol, respectively, as the principal aglycons found in significant concentrations (*10–12*). Moreover, these two aglycons were present in *N. officinale* with more quercetin than kaempferol (*13, 14*). In general, these aglycons are glycosylated and acylated, and these changes in their structures could confer different biological properties. Nevertheless, the polyphenol profiles of watercress and mizuna had not been characterized before.

The vitamin C content of these baby leaf species is important as an antioxidant source in the diet. In previous studies the content of vitamin C reported in *E. sativa* and *N. officinale* was higher than in other salad ingredients, for example, different varieties of *Lactuca sativa*, such as red lettuce and iceberg (*14, 15*). The antioxidant capacity has been evaluated in *D. tenuifolia*

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and *E. vesicaria* (*16*) as well as in *E. sativa* and *N. officinale* (*15*). These reports showed that the antioxidant capacity depends on the species, the method used, and the extraction of plant material.

The main objective of the present work was the comparative study of antioxidant compounds, polyphenols and vitamin C, in cruciferous vegetables used as salad ingredients: wild rocket [*Diplotaxis tenuifolia* (L.) DC.], salad rocket [*Eruca vesicaria* (L.) Cav.], mizuna [*Brassica rapa* L. subsp. *nipposinica* (L.H. Bailey) Haneltand], and watercress (*Nasturtium officinale* R. Br.). Moreover, in this study, polyphenolic compounds were characterized by HPLC-MS in mizuna and watercress. The possible relationships between chemical composition and antioxidant capacity of these cruciferous salad vegetables were also evaluated.

MATERIALS AND METHODS

Plant Material. The species mizuna [*Brassica rapa* L. subsp. *nipposinica* (L.H. Bailey) Haneltand], watercress (*Nasturtium officinale* R. Br.), wild rocket [*Diplotaxis tenuifolia* (L.) DC.], and salad rocket [*Eruca vesicaria* (L.) Cav.] were cultivated in winter in fields located in La Aparecida (Murcia, Spain) and supplied by Agrolito S.L. (Torre Pacheco, Murcia, Spain). *Brassicaceae* leaves at commercial stage were harvested in March and immediately transported to the laboratory (Espinardo, Murcia, Spain). Approximately three replicates of 300 g of each plant, free from decay and/or mechanical damage, were selected at random. Fresh samples were used to evaluate the content of vitamin C and polyphenols as well as the antioxidant capacity. Freeze-dried material was used for the identification of polyphenols.

Isolation and Identification of Polyphenolic Compounds. The identification of polyphenols in mizuna and watercress leaves was performed according to the methodology previously described for wild rocket and salad rocket by Martínez-Sánchez et al. (*12*).

Extraction. A freeze-dried sample (0.5 g) was macerated overnight at room temperature with methanol–water (5 mL, 1:1 v/v). The resulting extract was centrifuged (10500*g*) for 5 min and the supernatant collected and filtrated.

Alkaline Hydrolysis. Alkaline hydrolysis was achieved to obtain preliminary information of the structures of the flavonoid glycosides deacylated before the analysis of the crude extracts. One milliliter of 4 N NaOH was added to 1 mL of the extracts and was kept for 16 h in a stoppered test tube under N_2 atmosphere. The saponificated extracts were acidified with concentrated HCl until pH 1–2 and directly analyzed by HPLC-DAD-MS/MS (*17*).

LC/UV-DAD/ESI-MS^n Analyses. Chromatographic analyses were carried out on a LiChroCART column (250 × 4 mm, RP-18, 5 μm particle size, LiChrospher100 stationary phase; Merck, Darmstadt, Germany) protected with a LiChroCART guard column (4 × 4 mm, RP-18, 5 μm particle size; Merck, Darmstadt, Germany). The mobile phase consisted of two solvents: water−formic acid (0.1%) (A) and methanol (B). For the analysis of both free flavonol glycosides and the corresponding acylated derivatives, a linear gradient starting with 20% B was installed to reach 50% B at 30 min. The flow rate was 1 mL min^{-1}, and the injection volume was 20 μL. Spectral data from all peaks were accumulated in the range 240–400 nm. The chromatograms were recorded at 330 nm. The HPLC/UV-DAD/ESI-MS^n analyses were carried out in an Agilent HPLC 1100 series equipped with a diode array detector and mass detector in series (Agilent Technologies, Waldbronn, Germany). The HPLC consisted of a binary pump (model G1312A), an autosampler (model G1313A), a degasser (model G1322A), and a photodiode array detector (model G1315B). The HPLC system was controlled by ChemStation software (Agilent, v.08.03). The mass detector was an ion trap spectrometer (model G2445A) equipped with an electrospray ionization interface and was controlled by LCMSD software (Agilent, v.4.1). The ionization conditions were adjusted at 350 °C and 4 kV for capillary temperature and voltage, respectively. The nebulizer pressure and flow rate of nitrogen were 65.0 psi and 11.0 L min^{-1}, respectively. The full scan mass covered the range from *m*/*z* 200 to *m*/*z* 2000 for free glycosides and acylated derivatives and from *m*/*z* 90 to *m*/*z* 400 for acids and aglycons. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 to 2 V. Mass spectrometry data were acquired in the negative ionization mode. MS^n was carried out in the automatic mode on the more abundant fragment ion in MS^{n-1}.

Quantitative Analysis of Polyphenolic Compounds. Fresh sampled leaves cut into pieces (10 g fresh weight) were homogenized with 20 mL of of methanol–water (5:95) plus citric acid (21.0 g L^{-1}) and EDTA (0.5 g L^{-1}). The homogenates were filtered through cheesecloth and then through a 0.45 μm pore filter (Millex HV13; Millipore, Bedford, MA). The HPLC analyses of polyphenols were performed according to the methodology reported by Martínez-Sánchez et al. (*18*) with some modifications. Samples of 20 μL were analyzed, and chromatograms were recorded at 330 nm. The sinapic acid derivatives and flavonols were characterized according to UV spectra and quantified by comparison with external standards of sinapic acid and rutin (Sigma, St. Louis, MO) (*4*). Results are expressed in milligrams per 100 g fresh weight (fw).

Analysis of Vitamin C. The same extract used for the analysis of polyphenols was employed for the analysis of vitamin C. Fresh sampled leaves cut into pieces (10 g fw) were homogenized with 20 mL of methanol–water (5:95) plus citric acid (21.0 g L^{-1}) and EDTA (0.5 g L^{-1}) (*18*). The homogenates were filtered through cheesecloth and then through a 0.45 μm pore filter (Millex HV13; Millipore, Bedford, MA). The extract was filtered through cheesecloth and a C_{18} Sep-Pak cartridge (Waters, Milford, MA), and ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined as described by Zapata and Dufour (*19*). HPLC analyses were performed after derivatization of DHA into the fluorophore 3-(1,2-dihydroxyethyl)furol[3,4-*b*]quinoxalin-1-one (DFQ) with 1,2-phenylenediamine dihydrochloride (OPDA). Samples of 20 μL were analyzed with Merck-Hitachi (Tokyo, Japan) HPLC equipped with a L-4000 UV detector and a L-6000 pump. Separations of DFQ and AA were achieved on a Kromasil 100 C_{18} column (250 mm × 4 mm; 5 μm particle size; Tecnokroma, Barcelona, Spain). The mobile phase was MeOH/H_2O (5:95 v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate at pH 4.5. The flow rate was 0.9 mL min^{-1}. The detector wavelength was initially set at 348 nm, and after elution of DFQ, the wavelength was manually shifted to 261 nm for AA detection. Standard solution, column conditioning, and derivatization procedures have been previously described (*20*).

Antioxidant Activity. The influence of vitamin C and polyphenol content among other antioxidant compounds on the antioxidant activity was estimated in the same extract used for the analysis of polyphenols. The free radical scavenging capacity was evaluated by both ABTS and DPPH assays and the ferric reducing antioxidant power by the FRAP assay (*21*). All reactions started by adding 5 μL of the corresponding sample, and the final volume was 1 mL.

Statistical Analysis. Data represent the mean of three replicates per species. The values corresponding to polyphenol content were submitted to a factorial analysis of variance, and the mean values were compared by the least significant difference test (LSD). Bilateral correlations were determined by Pearson's correlation coefficient by statistical program SPSS (14.0).

RESULTS AND DISCUSSION

Flavonoid Compounds in Watercress Leaves. A large number of flavonoid glycosides were observed in the hydroalcoholic extract of watercress. Two types of UV spectra were distinguished: the typical UV spectra of flavonol 3-*O*-glycosides (*22*) corresponding to compounds **1**^{w}−**4**^{w} and **6**^{w} (**Figure 1**) and the typical UV spectra of cinnamoyl acylated derivatives corresponding to compounds **14**^{w}−**36**^{w} (**Figure 1B**). In the last case, their UV spectral shape resembles the overlapping of a flavonol spectrum with one hydroxycinnamic acid, with a broad maximum around 310–330 nm and a short maximum or shoulder around 255–271 nm, which, therefore, can be erroneously characterized as a cinnamic acid derivative (*5*). Previous

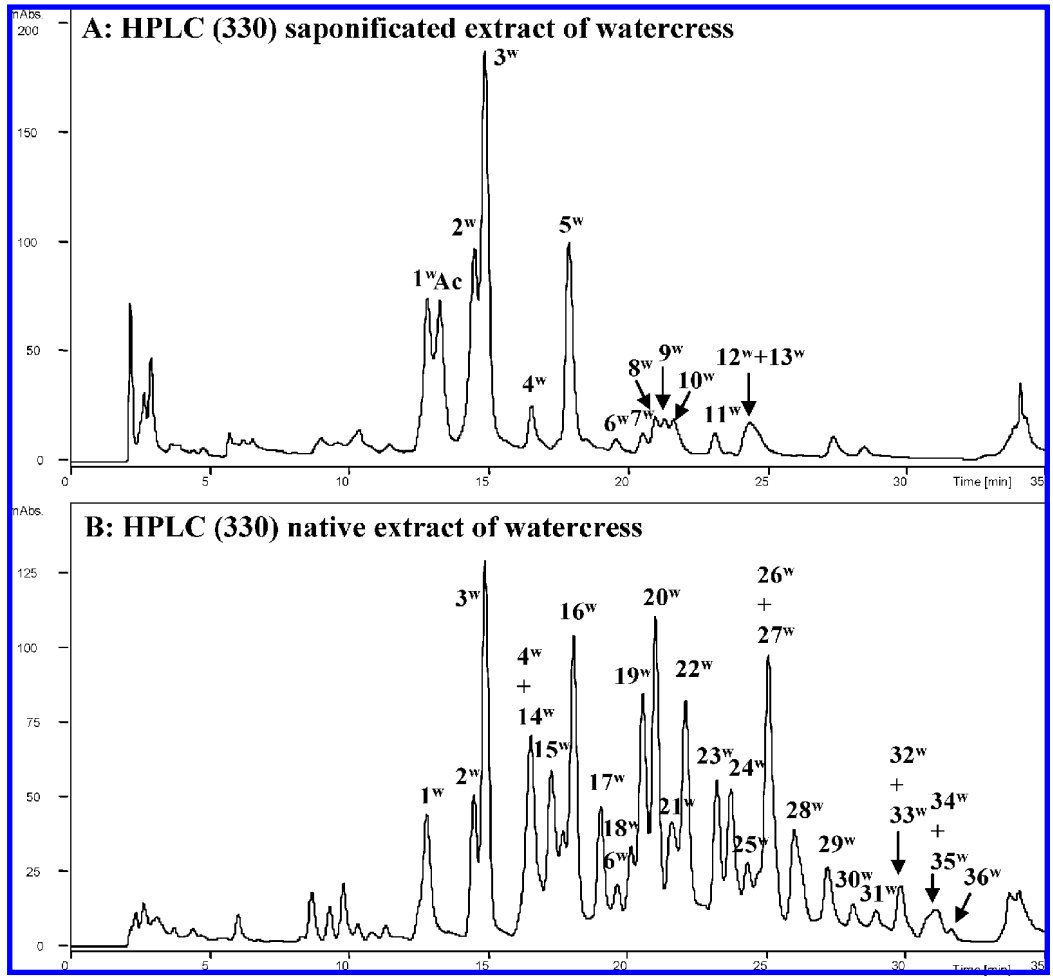

Figure 1. HPLC-DAD chromatogram of watercress flavonoids: (**A**) saponificated extract and (**B**) native extract.

to the characterization of polyphenols in the native extract, the deacylated compounds were studied by HPLC-DAD-MS/MS after alkaline hydrolysis of the raw extract.

Deacylated Compounds. The UV study of the saponificated extract revealed the presence of 13 flavonol 3-*O*-glycosides (**Figure 1A**, **Table 1**). The UV and MS analysis of these compounds showed the occurrence of quercetin and kaempferol derivatives with a high degree of glycosylation (tri- and tetraglycosides) and lower rate of diglycosides (**Table 1**). The (-MS2[M − H]-) event of compounds **1**^{w}−**4**^{w}, **6**^{w}, and **9**^{w} showed a loss of 146 mu obtaining the almost exclusive base peak ($Y^{7}{}_{0}{}^{-}$) which revealed the presence of rhamnose at the 7 position and dihexoside or trihexosides at the 3 position (*17*). On the other hand, the type of fragmentation of the diglycosidic fraction at the 3 position (-MS3[(M − H) → (M − H − 146)]$^{-}$) in the isomers **2**^{w}/**6**^{w}, **4**^{w}/**9**^{w}, and **10**^{w}/**13**^{w} (**Table 1**) revealed an interglycosidic linkage 1→2 in **2**^{w}, **4**^{w}, and **10**^{w} and 1→6 in **6**^{w}, **9**^{w}, and **13**^{w} (*17*). Similar structures have been previously reported in *Brassicaceae* such as cauliflower (*4*), broccoli (*5*), and tronchuda cabbage (*23*) with hexose as the only sugar moiety. In the case of watercress, thhe rhamnose moiety was detected at the 7 position in contrast with the other *Brassicaceae* species.

The tentative structures of flavonol glycosides characterized after alkaline hydrolysis are quercetin 3-*O*-triglucoside-7-*O*-rhamnoside (**1**^{w}), quercetin 3-*O*-sophoroside-7-*O*-rhamnoside (**2**^{w}), kaempferol 3-*O*-triglucoside-7-*O*-rhamnoside (**3**^{w}), kaempferol 3-*O*-sophoroside-7-*O*-rhamnoside (**4**^{w}), kaempferol 3-*O*-triglucoside (**5**^{w}), quercetin 3-*O*-gentiobioside-7-*O*-rhamnoside (**6**^{w}), kaempferol 3-*O*-gentiobioside-7-*O*-rhamnoside (**9**^{w}), kaempferol 3-*O*-sophoroside (**10**^{w}), and kaempferol 3-*O*-gentiobioside (**13**^{w}) (**Table 1**). In addition, two diglycoside derivatives of quercetin and two of kaempferol were tentatively identified (**7**^{w}, **8**^{w}, **11**^{w}, and **12**^{w}, respectively). According to MS fragmentation data, these flavonols showed glycosylation with hexose (probably glucose) and rhamnose bonds at different phenolic hydroxyls of the flavonol skeleton (**Table 1**) (*17*).

Acylated Compounds. In crude extracts other cinnamoyl acylated derivatives (**14**^{w}−**36**^{w}) were detected (**Figure 1B**, **Table 2**). The MS fragmentation patterns of these acylated derivatives were similar to those previously reported in broccoli (*5*) and tronchuda cabbage (*23*). After -MS2[M − H]- fragmentation of these compounds, a common loss of 146 mu (rhamnosyl moiety), which led to the base peak, was detected as that found in the deacylated compounds. These data suggested that the acylation was not on the sugar moiety. Other fragment ions from the loss of the acyl and/or this radical plus rhamnosyl were also detected (**Table 2**). The -MS3[(M − H) → (M − H − Rhmn)]$^{-}$ fragmentation of this base peak showed the loss of the acyl moiety leaving the aglycon fragment linked to a glycosidic fraction at the 3 position as the base peak. Concerning the deacylated derivatives (**29**^{w}−**36**^{w}), a new MS event (-MS4[(M − H) → (M − H − Rhmn) → (M − H − Rhmn − Acyl)]$^{-}$) was conducted for the delivery of the referred fragment in the case of diacylated derivatives (**Table 2**). Among these compounds, couples of isomers (**14**^{w}/**15**^{w}, **21**^{w}/**25**^{w}, **24**^{w}/**26**^{w}, **27**^{w}/**28**^{w}, **29**^{w}/**30**^{w}, **33**^{w}/**34**^{w}, and **35**^{w}/**36**^{w}) were observed due to the

Table 1. t_R, UV, and -MS: $[M - H]^-$, -MS2$[M - H]^-$, and -MS3$[(M - H) \rightarrow (M - H - 146)]^-$ Data of Flavonol Glycoside Derivatives from Saponification of Hydroalcoholic Extract of Watercress[a]

no.	compound[b]	t_R (min)	UV (nm)	$[M - H]^-$ (*m/z*)	-MS2$[M - H]^-$ (*m/z*) (%)	-MS3$[(M - H) \rightarrow (M - H - 146)]^-$ (*m/z*) (%)				
								Flav 3-triGlc-7-Rhmn		
					$Y^7_0{}^-$ (−146)	$[Y^7_0\,Y^3_2]^-$ (−162)	$[Y^7_0\,Z^3_2]^-$ (−180)	$[Y^7_0{}^{,2}X^3_1]^-$ (−162–120)	$[Y^7_0\,Z^3_1]^-$ (−162–180)	$[Y^7_0\,Y^3_0]^-$ (−486)
1[w]	Querc 3-triGlc-7-Rhmn	12.7	257, 267 sh, 355	933	787 (100)	625 (15)		505 (17)		301 (100)
3[w]	Kaempf 3-triGlc-7-Rhmn	14.7	265, 351	917	771 (100)	609 (13)	591 (50)	489 (25)	429 (13)	285 (100)
								Flav 3-diGlc-7-Rhmn		
						$[Y^7_0{}^{,2}X^3_1]^-$ (−120)	$[Y^7_0\,Y^3_1]^-$ (−162)		$[Y^7_0\,Z^3_1]^-$ (−180)	$[Y^7_0\,Y^3_0]^-$ (−324/325)
2[w]	Querc 3-diGlc-7-Rhmn	14.3	257, 267 sh, 353	771	625 (100)	505 (20)	463 (19)		445 (35)	300 (100)
6[w]	Querc 3-diGlc-7-Rhmn (isomer)	19.4	255, 265 sh, 355	771	625 (100)					301 (100)
4[w]	Kaempf 3-diGlc-7-Rhmn	16.4	265, 349	755	609 (100)	489 (10)			429 (55)	285 (100)
9[w]	Kaempf 3-diGlc-7-Rhmn (isomer)	21.1	266, 349	755	609 (100)					285 (100)
						Other Flav 3-glycosides				
								-MS2$[M - H]^-$		
						$Y^3_2{}^-$ (−162)	$Z^3_2{}^-$ (−180)		$Y^3_1{}^-$	$Y^3_0{}^-$
5[w]	Kaempf 3-triGlc	17.7	265, 351	771		609 (45)	591 (25)			285 (100)
10[w]	Kaempf 3-diGlc	21.5	267, 347	609					447 (42)	285 (100)
13[w]	Kaempf 3-diGlc (isomer)	24.3	coelution with **12**	609					447 (9)	285 (100)
								-Rhmn (−146)	-Glc (−162)	
7[w]	Querc X-Glc-Y-Rhmn	20.4	255, 265 sh, 353	609				463 (60)	447 (100)	301 (78)
8[w]	Querc X-Glc-Y-Rhmn (isomer)	20.8	255, 265 sh, 355	609				463 (80)	447 (85)	301 (100)
11[w]	Kaempf X-Glc-Y-Rhmn	22.9	265, 349	593				447 (100)	431 (2)	285 (5)
12[w]	Kaempf X-Glc-Y-Rhmn (isomer)	24.1	coelution with **13**	593				447 (100)	431 (37)	285 (50)

[a] Principal fragments observed. Other ions were found, but they have not been included. [b] Abbreviations: Querc, quercetin; Kaempf, kaempferol; Rhmn, rhamnoside; Glc, glucoside.

Table 2. t_R, UV, and -MS: $[M - H]^-$, -MS2$[M - H]^-$, -MS3$[(M - H) \rightarrow (M - H - 146)]^-$, and -MS4$[(M - H) \rightarrow (M - H - 146) \rightarrow (M - H - 146 - Acyl)]^-$ Data of Acylated Derivatives from Flavonoid 3-*O*-Diglucoside/Triglucoside-7-*O*-Rhamnoside of Watercress

					MS2$[M - H]^-$ (*m/z*) (%)								-MS3$[(M - H) \rightarrow (M - H - 146)]^-$ (*m/z*) (%)								-MS4$[\rightarrow \rightarrow (M - H - 146 - Acyl)]^-$ (*m/z*) (%)		
no.	compound[a]	t_R (min)	UV (nm)	$[M - H]^-$ (*m/z*)	−146 -Rhmn	−162 -Caf	−176 -Fer	−206 -Sinp	−292 (-R-*p*.C)	−308 (-R-C)	−322 (-R-F)	−352 (-R-S)	−146 -*p*.Coum	−162 -Caf	−176 -Fer	−206 -Sinp	−308 (-*p*.C-C)	−322 (-*p*.C-F)	−352 (-*p*.C-S)	−352 (-F-F)	−162 -Caf	−176 -Fer	−206 -Sinp
14[W]	1-Caf	16.4	—[b]	1095	949 (100)	933 (33)				787 (90)				787 (100)									
15[W]	1-Caf (isomer)	17.1	250, 271 sh, 309 sh, 329	1095	949 (100)	933 (20)				787 (60)				787 (100)									
16[W]	2-Caf	17.9	255, 267 sh, 309 sh, 331	933	787 (100)	771 (70)				625 (40)				625 (100)									
17[W]	3-Caf	18.9	269, 329	1079	933 (100)					771 (5)				771 (100)									
18[W]	4-Caf	20.0	265, 283 sh, 333	917	771 (100)					609 (7)				609 (100)									
19[W]	1-Sinp	20.4	—[b]	1139	993 (100)			933 (14)				787 (18)				787 (100)							
20[W]	6-Sinp	20.8	—[b]	977	831 (100)			771 (40)				625 (30)				625 (100)							
21[W]	1-Fer	21.5	255, 263 sh, 307 sh, 337	1109	963 (100)		933 (7)				787 (15)				787 (100)[c]								
22[W]	3-Sinp	21.9	265, 305 sh, 331	1123	977 (100)			771 (2)				771 (2)				771 (100)[c]							
23[W]	3-Fer	23.1	267, 310 sh, 343	1093	947 (100)						771 (2)				771 (88)[c]								
24[W]	1-*p*.Coum	23.6	258, 269 sh, 317	1079	933 (100)				787 (60)				787 (100)										
25[W]	1-Fer (isomer)	24.2	—[b]	1109	963 (100)		933 (7)				787 (15)				787 (100)[c]								
26[W]	1-*p*.Coum (isomer)	24.7	—[b]	1079	933 (100)				787 (44)				787 (100)										
27[W]	3-*p*.Coum	24.9	—[b]	1063	917 (100)				771 (17)				771 (100)										
28[W]	3-*p*.Coum (isomer)	25.8	267, 319	1063	917 (100)				771 (14)				771 (100)										
29[W]	3-*p*.Coum/Caf	27.1	270, 321	1225	1079 (100)				933 (26)	917 (5)			933 (100)				771 (25)				771 (100)		
30[W]	3-*p*.Coum/Caf (isomer)	28.0	269, 327	1225	1079 (100)				933 (40)	917 (3)			933 (100)				771 (29)				771 (100)		
31[W]	1-*p*.Coum/Sinp	28.8	271, 323	1285	1139 (100)			1079 (11)	993 (45)				993 (100)						787 (21)				787 (100)
32[W]	1-Fer/Fer	29.6	—[b]	1285	1139 (100)		1109 (27)				963 (47)				963 (100)					787 (20)		787 (100)	
33[W]	1-*p*.Coum/Fer	29.6	—[b]	1255	1109 (100)		1079 (6)		963 (53)		933 (13)		963 (100)		933 (22)			787 (20)				787 (100)	
34[W]	1-*p*.Coum/Fer (isomer)	30.7	—[b]	1255	1109 (100)		1079 (6)		963 (42)		933 (14)		963 (100)		933 (14)			787 (26)				787 (100)	
35[W]	3-*p*.Coum/Fer	31.0	—[b]	1239	1093 (100)				947 (16)				947 (100)		917 (15)			771 (11)				771 (100)	
36[W]	3-*p*.Coum/Fer (isomer)	31.5	—[b]	1239	1093 (100)				947 (32)				947 (100)					771 (83)				771 (100)	

[a] Abbreviations: R (Rhmn), rhamnosyl; *p*.C (*p*.Coum), *p*-coumaroyl; F (Fer), feruloyl; S (Sinp), sinapoyl; C (Caf), caffeoyl. Key: 1, Querc 3-triGlc-7-Rhmn; 2, Querc 3-diGlc-7-Rhmn; 3, Kaempf 3-triGlc-7-Rhmn; 4, Kaempf 3-diGlc-7-Rhmn; 6, Querc 3-diGlc-7-Rhmn (isomer). [b] UV spectra are not shown due to the visualization difficulties because of coeluting peaks. [c] After the MS3 event of ferulic and sinapic acid derivatives, a high abundant ion with 14 u higher than the fragment obtained by this acyl loss can be observed [compounds, *m/z* (%): **21**, 801 (8); **22**, 785 (40); **23**, 785 (100); **25**, 801 (53)].

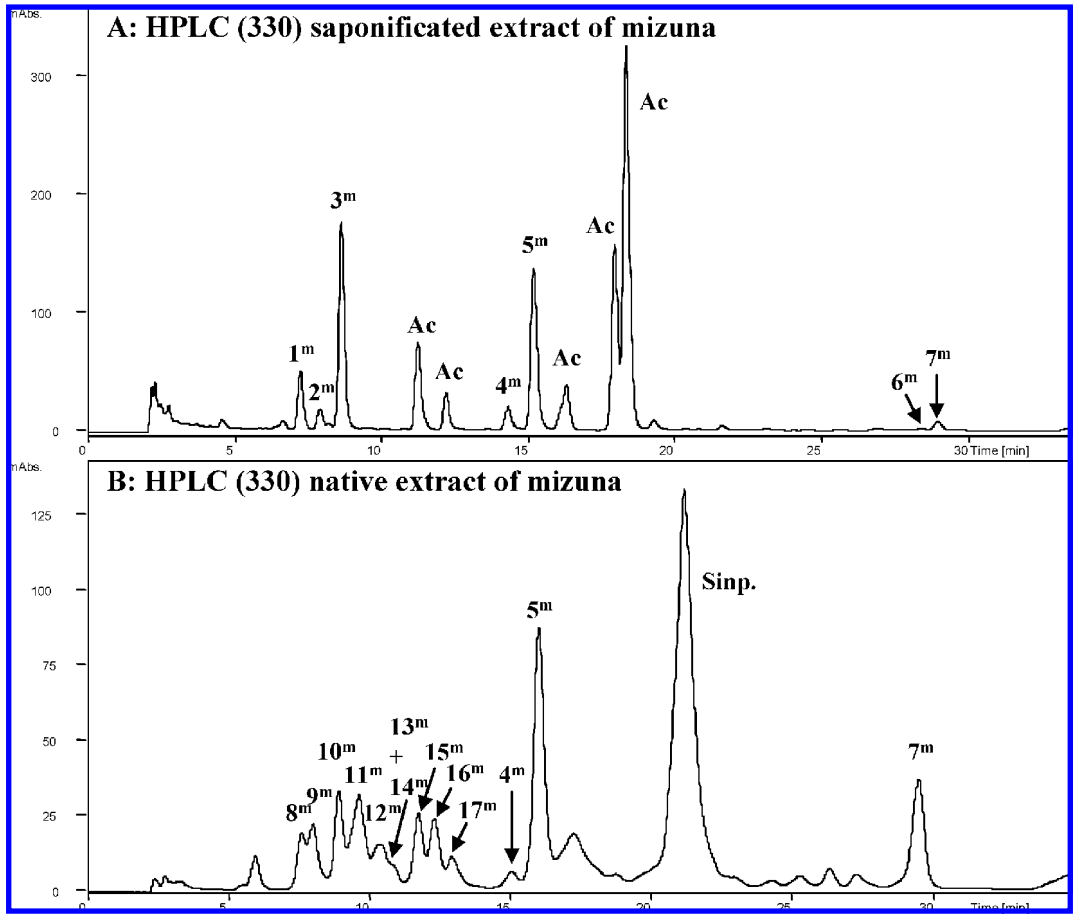

Figure 2. HPLC-DAD chromatogram of mizuna polyphenols: (**A**) saponificated extract and (**B**) native extract.

different position of the acyl moiety on the glycosidic moiety at the 3 position.

After the study of the hydrolyzed and nonhydrolyzed extracts, flavonols $\mathbf{1}^{w}-\mathbf{4}^{w}$ and $\mathbf{6}^{w}$ and the corresponding acylated forms were observed. The other deacylated derivatives of flavonoids were presented in trace amounts, or they can be artefacts generated during the saponification process.

Flavonoid Compounds in Mizuna Leaves. The analysis of the mizuna hydroalcoholic extract showed an intense sinapic acid peak (**Figure 2B**), which had the following characteristics (UV, 326 nm; -MS, 223 $[M - H]^-$; -MS2$[M - H]^-$): 208 (25%, $[M - H - 15]^-$), 179 (50%, $[M - H - 44]^-$), 164 (100%, $[M - H - 15 - 44]^-$), 149 (25%, $[M - H - 15 - 15 - 44]^-$). Other compounds with flavonoid UV spectra such as $\mathbf{4}^{m}$, $\mathbf{5}^{m}$, and $\mathbf{7}^{m}$ and cinnamoyl acylated derivatives of flavonoids $\mathbf{8}^{m}-\mathbf{17}^{m}$ were also observed (**Figure 2B**).

In this study, the saponification was also assayed in order to elucidate the flavonoid structures of the deacylated compounds as a previous step for the elucidation of the acylated compounds. The HPLC chromatogram of the saponificated extract showed the presence of seven flavonoids ($\mathbf{1}^{m}-\mathbf{7}^{m}$) and other compounds with UV spectra of hydrocynnamic acid (**Figure 2A**). The MS fragmentation of compounds $\mathbf{1}^{m}-\mathbf{3}^{m}$ was typical of flavonol 3-*O*-di/trihexoside-7-*O*-hexoside (**Table 3**) (*17*). These compounds and/or their cinnamoyl derivatives are widely distributed in *Brassicaceae* as the main compounds. Thus 3-*O*-di/triglucoside 7-*O*-glucoside derivatives of kaempferol and quercetin have been identified in cauliflower (*4*), broccoli (*5*), and tronchuda cabbage (*23*). Another flavonoid glycoside observed in both saponificated and native extracts ($\mathbf{5}^{m}$) was identified by MS analysis as isorhamnetin dihexoside (**Table 3**). Its MS fragmentation was in accordance with isorhamnetin with sugar moieties at different phenolic hydroxyls, particularly at the 3 position (353 nm, UV spectra) while other substitutions may be at the 7 or 4′ position (*17*) (**Table 3**). Isorhamnetin 3,4′-diglucoside has been characterized in rocket species (*12*). However, in mizuna and due to biosynthetic reasons, it is probable to find a flavonoid 3,7-di-*O*-hexoside. Therefore, compound $\mathbf{5}^{m}$ can be tentatively identified as isorhamnetin 3,7-di-*O*-glucoside and compound $\mathbf{4}^{m}$ as kaempferol 3,7-di-*O*-glucoside. In addition, kaempferol 3-*O*-glucoside ($\mathbf{6}^{m}$) and isorhamnetin 3-*O*-glucoside ($\mathbf{7}^{m}$) were detected in trace amounts.

Acylated Compounds. In the crude hydroalcoholic extract besides $\mathbf{4}^{m}$, $\mathbf{5}^{m}$, and $\mathbf{7}^{m}$ deacylated compounds, a group of acylated compounds were also detected ($\mathbf{8}^{m}-\mathbf{17}^{m}$) (**Figure 2B**, **Table 4**). The fragmentation of these compounds was similar to that indicated in watercress. The only difference concerned the base peak which was produced during the MS2 experiment as 162 mu loss instead of 146 mu loss. This led to glycosylation with hexose at the 7 position instead of a rhamnose. The cinnamoyls derived from compounds $\mathbf{1}^{m}$ (quercetin 3-*O*-diGlc-7-*O*-Glc) and $\mathbf{3}^{m}$ (kaempferol 3-*O*-diGlc-7-*O*-Glc) are widely distributed in *Brassicaceae* as well as other cinnamoyl derivatives. Therefore, isorhamnetin 3,7-di-*O*-diglucoside can be considered a marker to differentiate chemiotaxonomically mizuna species from other *Brassicaceae* species. In agreement with our finding, this compound was described in pack choi leaves [*Brassica rapa* L. ssp. *chinensis* L. (Hanelt)] (*6*), *Brassica rapa* L. ssp. *sylvestris* L. (*1*), *Brassica rapa* flower (*24*), and *Brassica rapa* var. *rapa* L. leaves, stems, and flower buds (*25*).

This work completes a previous analytical study on the polyphenols of wild rocket and salad rocket as new cruciferous

Table 3. t_R, UV, and -MS: $[M - H]^-$, -MS2$[M - H]^-$, and -MS3$[(M - H) \rightarrow (M - H - 162)]^-$ Data of Flavonol Glycoside Derivatives from Saponification of Hydroalcoholic Extract of Mizuna[a]

no.	compound[b]	t_R (min)	UV (nm)	$[M - H]^-$ (*m/z*)	-MS2$[M - H]^-$ (*m/z*) (%)		-MS3$[(M - H) \rightarrow (M - H - 162)]^-$ (*m/z*) (%)			
					$Y^7_0{}^-$ (−162/163)		$[Y^7_0\, Y^3_2]^-$ (−162/163)		$[Y^7_0\, Z^3_1]^-$ (−162–180)	$[Y^7_0\, Y^3_0]^-$ (−486)
2[m]	Kaempf 3-triGlc-7-Glc	7.7	265, 347	933	771 (100)		609 (10)		420 (40)	285 (100)
						(−324)	$[Y^7_0\, Y^3_1]^-$ (−162)	$[Y^7_0\, Z^3_1]^-$(−180)		$[Y^7_0\, Y^3_0]^-$ (−324/325)
1[m]	Querc 3-diGlc-7-Glc	7.1	257, 265 sh, 353	787	625 (100)		463 (10)	445 (20)		300 (100)
3[m]	Kaempf 3-diGlc-7-Glc	8.7	265, 349	771	609 (100)		447 (60)			284 (100)
4[m]	Kaempf 3-Glc-7-Glc	14.2	265, 347	609	447 (100)	285 (25)				284 (100)
5[m]	Isorhamn 3-Glc-7-Glc	15.0	255, 265 sh, 353	639	477 (100)	315 (15)				314 (100)
					$Y^3_0{}^-$					
6[m]	Kaempf 3-Glc	28.3	265, 347	447	284 (100)					
7[m]	Isorhamn 3-Glc	28.8	255, 267 sh, 353	477	314 (100)					

[a] Principal fragments observed. Other ions were found, but they have not been included. [b] Abbreviations: Querc, quercetin; Kaempf, kaempferol; Isorhamn, isorhamnetin; Glc, glucoside.

Table 4. t_R, UV, and -MS: $[M - H]^-$, -MS2$[M - H]^-$, and -MS3$[(M - H) \rightarrow (M - H - 162)]^-$ Data of Acylated Derivatives from Flavonoid 3-*O*-Diglycoside-7-*O*-Glucoside of Mizuna

					-MS2$[M - H]^-$ (*m/z*) (%)										-MS3$[(M - H) \rightarrow (M - H - 162)]^-$ (*m/z*) (%)				
no.	compound[a]	t_R (min)	UV (nm)	$[M - H]^-$ (*m/z*)	−146 -*p*.Coum	−162 -Glc	−176 -Fer	−192 -MCaf	−206 -Sinp	−308 (-G-*p*.C)	−324 (-G-C)	−338 (-G-F)	−354 (-G-MC)	−368 (-G-S)	−146 -*p*.Coum	−162 -Caf	−192 -MCaf	−176 -Fer	−206 -Sinp
8[m]	1-MCaf	7.5	250, 269 sh, 333	979		817 (90)		787 (100)					625 (60)				625 (100)		
9[m]	1-Caf	7.9	253, 269 sh, 299 sh, 334	949		787 (100)					625 (30)					625 (100)			
10[m]	3-MCaf	8.8	269, 331	963		801 (100)		771 (4)					609 (6)				609 (100)		
11[m]	3-Caf	9.5	267, 333	933		771 (100)					609 (5)					609 (100)			
12[m]	1-Sinp	10.1	250, 269 sh, 335	993		831 (100)			787 (50)					625 (35)					625 (100)[c]
13[m]	1-Fer	10.4	—[b]	963		801 (100)	787 (30)					625 (27)						625 (100)[c]	
14[m]	1-*p*.Coum	10.6	—[b]	933	787 (6)	771 (100)				625 (20)					625 (100)				
15[m]	3-Sinp	11.6	269, 333	977		815 (100)			771 (20)					609 (5)					609 (100)[c]
16[m]	3-Fer	12.2	269, 333	947		785 (100)	771 (10)					609 (2)						609 (60)[c]	
17[m]	3-*p*.Coum	13.1	269, 318	917	771 (3)	755 (100)				609 (2)					609 (100)				

[a] Abbreviations: C (Caf), caffeoyl; MC (Mcaf), methoxycaffeoyl; *p*.C (*p*.Coum), *p*-coumaroyl; F (Fer), feruloyl; S (Sinp), sinapoyl. Key: 1, Querc 3-diGlc-7-Glc; 3, Kaempf 3-diGlc-7-Glc. [b] UV spectra are not shown due to the visualization difficulties because of coeluting peaks. [c] After the MS3 event of ferulic and sinapic acid derivatives, a high abundant ion with 14 u higher than the fragment obtained by this acyl loss can be observed [compound, *m/z* (%): **12**, 639 (16); **13**, 639 (70); **15**, 623 (74); **16**, 623 (100)].

Table 5. Content of Individual Polyphenols from Baby Leaf *Brassicaceae*

peak no.[a]	compound[b]	watercress	mizuna	wild rocket	salad rocket
1^{w}	Q 3-triGlc-7-Rhmn	7.8 ± 2.9			
2^{w}	Q 3-diGlc-7-Rhmn	7.3 ± 1.4			
3^{w}	K 3-triGlc-7-Rhmn	18.4 ± 3.7			
4^{w}	K 3-diGlc-7-Rhmn	8.3 ± 1.9			
14^{w}	Q 3-(Caf-triGlc)-7-Rhmn	10.1 ± 2.4			
15^{w} + **16**^{w}	Q 3-(Caf-triGlc)-7-Rhmn (isomer) + Q 3-(Caf-diGlc)-7-Rhmn	19.7 ± 4.0			
17^{w} + **6**^{w} + **18**^{w}	K 3-(Caf-triGlc)-7-Rhmn + Q 3-diGlc-7-Rhmn + K 3-(Caf-diGlc)-7-Rhmn	13.1 ± 3.0			
19^{w}	Q 3-(Sinp-triGlc)-7-Rhmn	14.1 ± 3.0			
20^{w} + **21**^{w}	Q 3-(Sinp-diGlc)-7-Rhmn (isomer) + Q 3-(Fer-triGlc)-7-Rhmn	29.3 ± 6.3			
22^{w}	K 3-(Sinp-triGlc)-7-Rhmn	10.2 ± 2.2			
23^{w} + **24**^{w}	K 3-(Fer-triGlc)-7-Rhmn + Q 3-(*p*.Coum-triGlc)-7-Rhmn	35.7 ± 7.5			
25^{w}	Q 3-(Fer-triGlc)-7-Rhmn (isomer)	14.8 ± 3.0			
26^{w}	Q 3-(*p*.Coum-triGlc)-7-Rhmn (isomer)	10.9 ± 2.2			
27^{w} + **28**^{w} + **29**^{w}	K 3-(*p*.Coum-triGlc)-7-Rhmn + K 3-(*p*.Coum-triGlc)-7-Rhmn (isomer) + K 3-(*p*.Coum/Caf-triGlc)-7-Rhmn	19.6 ± 4.4			
30^{w}	K 3-(*p*.Coum/Caf-triGlc)-7-Rhmn (isomer)	16.7 ± 3.4			
31^{w}	Q 3-(*p*.Coum/Sinp-triGlc)-7-Rhmn	9.9 ± 2.7			
32^{w}	Q 3-(Fer/Fer-triGlc)-7-Rhmn	3.8 ± 1.2			
33^{w}	Q 3-(*p*.Coum/Fer-triGlc)-7-Rhmn	4.2 ± 1.4			
34^{w}	Q 3-(*p*.Coum/Fer-triGlc)-7-Rhmn (isomer)	4.6 ± 2.4			
35^{w}	K 3-(*p*.Coum/Fer-triGlc)-7-Rhmn	2.3 ± 0.7			
36^{w}	K 3-(*p*.Coum/Fer-triGlc)-7-Rhmn (isomer)	1.7 ± 0.8			
	sinapic acid		22.5 ± 1.5		
8^{m}	Q 3-(Mcaf-diGlc)-7-Glc		2.3 ± 1.1		
9^{m}	Q 3-(Caf-diGlc)-7-Glc		2.5 ± 1.0		
10^{m}	K 3-(Mcaf-diGlc)-7-Glc		5.7 ± 1.2		
11^{m}	K 3-(Caf-diGlc)-7-Glc		9.4 ± 1.4		
12^{m}	Q 3-(Sinp-diGlc)-7-Glc		5.3 ± 0.4		
13^{m}	Q 3-(Fer-diGlc)-7-Glc		4.2 ± 1.3		
14^{m}	Q 3-(*p*.Coum-diGlc)-7-Glc		10.2 ± 1.1		
15^{m}	K 3-(Sinp-diGlc)-7-Glc		7.1 ± 0.1		
16^{m}	K 3-(Ferp-diGlc)-7-Glc		3.9 ± 0.6		
17^{m}	K 3-(*p*.Coum-diGlc)-7-Glc		2.4 ± 0.6		
4^{m}	K 3-(diGlc)-7-Glc		2.7 ± 0.1		
5^{m}	I 3-Glc-7-Glc		21.3 ± 1.2		
1^{wr}	Q 3,3′,4′-triGlc			43.5 ± 7.1	
3$^{wr/sr}$	K 3,4′-diGlc			3.8 ± 1.3	97.8 ± 13.3
4^{wr}	Q 3,4′-diGlc-3′-(6-MC-Glc)			1.5 ± 0.9	
5$^{wr/sr}$	I 3,4′-diGlc			4.5 ± 0.1	10.7 ± 1.4
6^{wr}	Q 3,4′-diGlc-3′-(6-Sinap-Glc)			42.2 ± 4.5	
7^{wr}	Q 3,4′-diGlc-3′-(6-Fer-Glc)			5.7 ± 1.3	
8^{wr}	Q 3,4′-diGlc-3′-(6-*p*.Coum-Glc)			1.6 ± 0.2	
9^{wr}	Q 3-(2-Mcaf-Glc)- 3′-(6-Sinp- Glc)-4′-Glc			1.4 ± 0.1	
10^{wr}	Q 3-(2-Caf-Glc)- 3′-(6-Sinp-Glc)-4′-Glc			2.3 ± 0.0	
11^{wr}	Q 3-(2-Sinp-Glc)- 3′-(6-Sinp-Glc)-4′-Glc			25.2 ± 0.7	
12^{wr}	Q 3-(2-Fer-Glc)- 3′-(6-Sinp-Glc)-4′-Glc			6.1 ± 0.4	
13^{wr}	Q 3-(2-Fer-Glc)- 3′-(6-Fer-Glc)-4′-Glc			1.2 ± 0.2	
14^{sr}	Q 3-Glc				9.1 ± 0.6
15^{sr}	K 3-Glc				3.3 ± 0.4
16^{sr}	I 3-Glc				8.3 ± 0.6
17^{sr}	K 3-(2-Sinp-Glc)-4′-Glc				3.1 ± 0.7
	total polyphenols[c]	262.7 ± 56.9	99.4 ± 1.1	139.1 ± 11.5	132.3 ± 17.1

[a] Peak number corresponds with the peaks in the chromatogram [**Figures 1** and **2** for watercress and mizuna and our previous work (*18*) for wild rocket and salad rocket] for each species. m = mizuna; w = watercress; wr = wild rocket; sr = salad rocket. Numbering of the wild rocket and salad rocket compounds followed the order described in our previous work (*12*). [b] Abbreviations: Caf, caffeoyl; Mcaf, methoxycaffeoyl; *p*.Coum, *p*-coumaroyl; Fer, feruloyl; Sinp, sinapoyl; Glc, glucosyl; Rhmn, rhamnosyl; Q, quercetin; K, kaempferol; I, isorhamnetin. [c] Values are means ± standard deviations, expressed as mg 100 g^{-1} fw.

species used for salads (*12*). In summary, flavonol derivatives of quercetin and kaempferol were detected in watercress, mizuna, wild rocket, and salad rocket. Quercetin derivatives were the main compounds in wild rocket, while kaempferol derivatives were the main compounds in salad rocket. The isorhamnetin derivatives found in wild rocket and salad rocket were also abundant in mizuna but not detected in watercress. In addition, the glycosylation pattern tentatively characterized in this study was different among the studied species. The glycosylation with glucose at the 3 position was common in all these species, while the second sugar moiety at the 7 position was only observed in watercress and mizuna, with the particularity that this sugar moiety is a rahmnose in watercress and glucose in mizuna. On the contrary, in wild rocket and salad rocket subsequent glycosylations with glucose at the 4′ and 3′ positions were detected.

Quantitative Analysis of Polyphenolic Compounds. The polyphenolic compounds of the four *Brassicaceae* leaves were quantified by HPLC/DAD. The different aglycons presented a characteristic abundance for each cruciferous species studied. Watercress leaves showed the highest polyphenol content among species tested provided by quercetin and kaempferol derivatives in similar proportions (**Table 5**). On the other hand, mizuna presented a balanced variety between nonflavonoids such as sinapic acid and flavonoid derivatives of isorhamnetin, quercetin, and kaempferol. The highest content of sinapic acid was present

in this cruciferous species (22 mg 100 g^{-1} fw). Moreover, isorhamnetin, kaempferol, and quercetin derivatives were also found in significant amounts (21, 31, and 24 mg 100 g^{-1} fw, respectively) (**Table 5**). The main compounds in mizuna leaves were isorhamnetin 3-*O*-glucoside-7-*O*-glucoside (**5**^{m}) and sinapic acid (Sinp.), each one being 23% of total phenol content. Wild rocket leaves showed three types of flavonols, quercetin, kaempferol, and isorhamnetin, although quercetin was the predominant flavonol (94% of total phenol content). One deacylated derivative of quercetin (**1**^{wr}) and two acylated derivatives of quercetin (**6**^{wr} and **11**^{wr}) were the most abundant flavonols (**Table 5**) (*12*). Concerning salad rocket leaves, kaempferol was the most abundant flavonoid (78.8%) compared to quercetin derivatives (6.9%) according to Martínez-Sánchez et al. (*12*) (**Table 5**). Focusing on the individual compounds, kaempferol 3,4′-di-*O*-glucoside was the main flavonoid in salad rocket leaves, representing 74% of total polyphenol content.

A high content of polyphenols with significant differences ($P \leq 0.001$) among the *Brassicaceae* leaves studied was observed. Thus, watercress leaves showed the highest content with mean values of 263 mg of polyphenols per 100 g fw, while for mizuna, salad rocket, and wild rocket leaves had the lowest content (99, 132, and 139 mg per 100 g fw, respectively) (**Table 5**). The polyphenol content of these new cruciferous vegetables incorporated in mixed salad leaves was higher than other salad ingredients such as some varieties of green lettuce (*26*). This content was also higher than the content reported for other *Brassicaceae* vegetables such as different broccoli cultivars, cabbage, Chinese cabbage, and Brusels sprouts (*7, 27, 28*). The different content of polyphenols may be influenced by several factors, including genetic and environmental influences, growing period, and maturity stage at harvest (*10, 28*).

Vitamin C. The vitamin C content measured as ascorbic acid (AA) and dehydroascorbic acid (DHAA) of *Brassicaceae* leaves ranges among 64–104 mg per 100 g fw. The highest content of vitamin C was observed in watercress (104 mg 100 g^{-1} fw consisting of 81 mg of AA plus 23 mg of DHAA) followed by wild rocket (103 mg 100 g^{-1} fw consisting of 73 mg of AA plus 30 mg of DHAA) and salad rocket (80 mg 100 g^{-1} fw consisting of 52 mg of AA plus 29 mg of DHAA). The lowest content of vitamin C was shown by mizuna (64 mg 100 g^{-1} fw consisting of 53 mg of AA plus 11 mg of DHAA).

AA was the predominant form of vitamin C in all of the cruciferous species studied. The highest content of AA was shown in leaves of watercress and wild rocket (81 and 73 mg 100 g^{-1} fw, respectively) while mizuna and salad rocket had the lowest (52 mg 100 g^{-1} fw). However, the percentage of both forms of vitamin C was different in the four *Brassicaceae* species tested. In this study, watercress and mizuna showed the highest proportion of AA form (78% and 83%, respectively). On the other hand, the highest percentage of DHAA form was observed in salad rocket leaves, reaching 35%.

The vitamin C content of these *Brassicaceae* species used as mixed salads was higher than other lettuce ingredients such as iceberg, lollo rosso, and chicory (4, 12, and 10 mg of vitamin C 100 g^{-1} fw, respectively), which are some of the most consumed vegetables in salads (*26*). These *Brassicaceae* vegetables had a similar content of vitamin C compared to spinach (*29*) and other *Brassicaceae* such as some broccoli cultivars (*27*). Moreover, previous work described differences in AA content of leaves because of both leaf age and the irradiance arriving at the leaf surface among other factors (*30*).

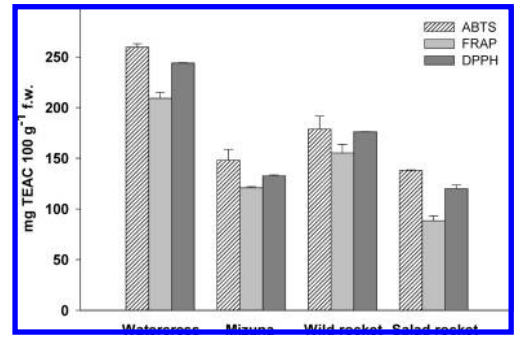

Figure 3. Antioxidant activity of *Brassicaceae* species by ABTS, FRAP, and DPPH assays.

Table 6. Correlation Coefficients of ABTS, FRAP, and DPPH To Evaluate the Antioxidant Activity in *Brassicaceae* Leaves

methods and antioxidant compounds[a]	Pearson's correlation coefficients
ABTS vs FRAP	0.95 [b]
ABTS vs DPPH	0.99 [b]
FRAP vs DPPH	0.98 [b]
vitamin C vs ABTS	0.72 [b]
vitamin C vs FRAP	0.70 [c]
vitamin C vs DPPH	0.77 [b]
flavonoids vs ABTS	0.86 [b]
flavonoids vs FRAP	0.77 [b]
flavonoids vs DPPH	0.86 [b]
kaempferol vs ABTS	0.19
kaempferol vs FRAP	−0.05
kaempferol vs DPPH	0.11
quercetin vs ABTS	0.78 [b]
quercetin vs FRAP	0.87 [b]
quercetin vs DPPH	0.85 [b]
isorhamnetin vs ABTS	−0.81 [b]
isorhamnetin vs FRAP	−0.86 [b]
isorhamnetin vs DPPH	−0.87 [b]

[a] Number of samples (*N*) was 12. [b] $P \leq 0.01$. [c] $P \leq 0.05$

Antioxidant Activity. The different methods tested showed diverse values of activity, reaching a similar value for each vegetable among the different methods assayed. The antioxidant activity by ABTS assay for individual species of salad *Brassicaceae* leaves ranged from 138 mg 100 g^{-1} fw in salad rocket to 260 mg 100 g^{-1} fw in watercress (**Figure 3**). The *Brassicaceae* leaves showed different ability to reduce the ferric ions. The highest FRAP value was detected in watercress extracts (209 mg 100 g^{-1} fw), while the lowest value was in salad rocket (88 mg 100 g^{-1} fw) (**Figure 3**). These results showed the same trend for ABTS as for FRAP assays. The antioxidant capacity of salad *Brassicaceae* leaves was similar using the DPPH method and the other methods. Watercress reached the highest value (244 mg 100 g^{-1} fw) whereas salad rocket showed the lowest value (120 mg 100 g^{-1} fw) (**Figure 3**).

Higher antioxidant activities were obtained with the ABTS method than with DPPH and FRAP methods. However, a high correlation was shown among the ABTS, FRAP, and DPPH values (**Table 6**).

The antioxidant activity of *Brassicaceae* leaves was highly correlated with the content of flavonoids and vitamin C, although Pearson's correlation coefficient was lower for vitamin C than for flavonoids (**Table 6**). Previous studies of the antiradical activity and polyphenol composition of broccoli, cabbage, and other green *Brassicaceae* found a correlation between antioxidant activity and total polyphenol content, with the exception of cauliflower (*31*). However, in addition to polyphenols, other constituents could exhibit antioxidant properties, for example, vitamins C and E as well as carotenoids (*7, 28*). Thus,

polyphenolic compounds and vitamin C are the major antioxidants of *Brassica* vegetables (*7*).

Regarding *Brassicaceae* flavonoids and antioxidant activity in this specific study, quercetin derivatives were well correlated with all methods while no significant correlations were found for kaempferol and isorhamnetin derivatives (**Table 6**). Antioxidant activity is directly linked to the particular structure of phenols. In fact, alterations in the arrangement of the hydroxyl groups and degree of substitution by glycosylation decrease the antioxidant activity (*3*).

The results indicate that watercress, wild rocket, mizuna, and salad rocket are good sources of antioxidants. However, there is a significant variation among species. Thus, watercress showed the highest content of polyphenol, with derivatives of quercetin and kaempferol. The highest content of vitamin C was observed in watercress and wild rocket. Moreover, quercetin derivatives were the principal polyphenol in wild rocket, while kaempferol derivatives were in salad rocket. Mizuna showed similar content of sinapic acids, isorhamnetin, quercetin, and kaempferol derivatives. These data show the potential value of salad *Brassicaceae* vegetables as a dietary source of antioxidants. Therefore, the variability in the composition and quantity of antioxidant compounds in the different leaves indicates the importance of eating a variety of fresh vegetables in every meal (*32*). It would be interesting to study the influence of different factors such as plant growth stage and harvesting time on the content of antioxidant compounds, because they can influence the contribution of antioxidant compounds of these vegetables in the diet.

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LITERATURE CITED

(1) Romani, A.; Vignlini, P.; Isolani, L.; Ieri, F.; Heimler, D. HPLC-DAD/MS characterization of flavonoids and hydroxycinnamic derivatives in turnip tops (*Brassica rapa* L. subsp. *sylvestris* L.). *J. Agric. Food Chem.* **2006**, *54*, 1342–1346.

(2) Kaur, C.; Kapoor, H. C. Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int. J. Food Sci. Technol.* **2002**, *37*, 153–161.

(3) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2* (4), 152–159.

(4) Llorach, R.; Gil-Izquierdo, A.; Ferreres, F.; Tomás-Barberán, F. A. HPLC-DAD-MS/MS ESI characterization of unusual highly glycosylated acylated flavonoids from cauliflower (*Brassica oleracea* L. var. *varbotrytis*) agroindustrial byproducts. *J. Agric. Food Chem.* **2003**, *51*, 3895–3899.

(5) Vallejo, F.; Tomás-Barberán, F. A.; Ferreres, F. Characterisation of flavonols in broccoli (*Brassica oleracea* L. var. *italica*) by liquid chromatography-UV diode-array detection-electrospray ionisation mass spectrometry. *J. Chromatogr. A* **2004**, *1054*, 181–193.

(6) Rochfort, S. J.; Imsic, M.; Jones, R.; Trenerry, V. C.; Tomkins, B. Characterization of flavonol conjugates in immature leaves of pack choi [*Brassica rapa* L. ssp. *chinensis* L. (Hanelt.)] by HPLC-DAD and LC-MS/MS. *J. Agric. Food Chem.* **2006**, *54*, 4855–4860.

(7) Podsedek, A. Natural antioxidants and antioxidant capacity of *Brassica* vegetables: A review. *LWT—Food Sci. Technol.* **2007**, *40*, 1–11.

(8) Satyan, K. S.; Swamy, N.; Dizon, D. S.; Singh, R.; Granai, C. O.; Brard, L. Phenethyl isothiocyanate (PEITC) inhibits growth of ovarian cancer cells by inducing apoptosis: Role of caspase and MAPK activation. *Gynecol. Oncol.* **2006**, *103*, 261–270.

(9) Higdon, J. V.; Delage, B.; Williams, D. E.; Dashwood, R. H. Cruciferous vegetables and human cancer risk: epidemiological evidence and mechanistic basis. *Pharmacol. Res.* **2007**, *55*, 224–236.

(10) Arabbi, P. R.; Genovese, M. I.; Lajolo, F. M. Flavonoids in vegetable foods commonly consumed in Brazil and estimated ingestion by the Brazilian population. *J. Agric. Food Chem.* **2004**, *52*, 1124–1131.

(11) Bennett, R. N.; Rosa, E. A. S.; Mellon, F. A.; Kroon, P. A. Ontogenic profiling of glucosinolates, flavonoids, and other secondary metabolites in *Eruca sativa* (salad rocket), *Diplotaxis erucoides* (wall rocket), *Diplotaxis tenuifolia* (wild rocket), and *Bunias orientalis* (Turkish rocket). *J. Agric. Food Chem.* **2006**, *54*, 40054015.4.

(12) Martínez-Sánchez, A.; Llorach, R.; Gil, M. I.; Ferreres, F. Identification of new flavonoid glycosides and flavonoid profiles to characterize rocket leafy salads (*Eruca vesicaria* and *Diplotaxis tenuifolia*). *J. Agric. Food Chem.* **2007**, *55*, 1356–1363.

(13) Justesen, U.; Knuthsen, P. Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in tradicional Danish dishes. *Food Chem.* **2001**, *73*, 245–250.

(14) Franke, A. A.; Custer, L. J.; Arakaki, C.; Murphy, S. P. Vitamin C and flavonoid levels of fruit and vegetables consumed in Hawaii. *J. Food Compos. Anal.* **2004**, *17*, 1–35.

(15) Hassimotto, N. M. A.; Genovese, M. I.; Lajolo, F. M. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *J. Agric. Food Chem.* **2005**, *53*, 2928–2935.

(16) Heimler, D.; Isolani, L.; Vignolini, P.; Tombelli, S.; Romani, A. Polyphenol content and antioxidative activity in some species of freshly consumed salads. *J. Agric. Food Chem.* **2007**, *55*, 1724–1729.

(17) Ferreres, F.; Llorach, R.; Gil-Izquierdo, A. Characterization of the interglycosidic linkage in di-, tri-, tetra- and pentaglycosylated flavonoids and differentiation of positional isomers by liquid chromatography/electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* **2004**, *39*, 312–321.

(18) Martínez-Sánchez, A.; Marín, A.; Llorach, R.; Ferreres, F.; Gil, M. I. Controlled atmosphere preserves quality and phytonutrients in wild rocket (*Diplotaxis tenuifolia*). *Postharvest Biol. Technol.* **2006**, *40*, 26–33.

(19) Zapata, S.; Dufour, J. F. Ascorbic, dehydroascorbic and isoascorbic acid simultaneous determinations by reverse phase ion interaction HPLC. *J. Food Sci.* **1992**, *57*, 506–511.

(20) Gil, M. I.; Ferreres, F.; Tomás-Barberán, F. A. Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *J. Agric. Food Chem.* **1999**, *47*, 2213–2217.

(21) Llorach, R.; Tomás-Barberán, F. A.; Ferreres, F. Lettuce and chicory byproducts as a source of antioxidant phenolic extracts. *J. Agric. Food Chem.* **2004**, *52*, 5109–5116.

(22) Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The systematic identification of flavonoids*; Springer: New York, 1970.

(23) Ferreres, F.; Valentão, P.; Llorach, R.; Pinheiro, C.; Cardoso, L.; Pereira, J. A.; Sousa, C.; Seabra, R. M.; Andrade, P. B. Phenolic compounds in external leaves of tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC). *J. Agric. Food Chem.* **2005**, *53*, 2901–2907.

(24) Sasaki, K.; Takahashi, T. A flavonoid from *Brassica rapa* flower as the UV-absorbing nectar guide. *Phytochemistry* **2002**, *61*, 339–343.

(25) Fernandes, F.; Valentao, P.; Sousa, C.; Pereira, J. A.; Seabra, R. M.; Andrade, P. B. Chemical and antioxidative assessment of dietary turnip (*Brassica rapa* var. *rapa* L.). *Food Chem.* **2007**, *105*, 1003–1010.

(26) Llorach, R.; Martínez-Sánchez, A.; Tomás-Barberán, F. A.; Gil, M. I.; Ferreres, F. Characterization of polyphenols and antioxidant properties of five lettuce varieties and escarole. *Food Chem.* **2008**, . *108*, 1028–1038.

(27) Vallejo, F.; Tomás-Barberán, F.; García-viguera, C. Potencial bioactive compounds in health promotion from broccoli cultivars grown in Spain. *J. Sci. Food Agric.* **2002**, *82*, 1293–1297.

(28) Singh, J.; Upadhyay, A. K.; Prasad, K.; Bahadur, A.; Rai, M. Variability of carotenes, vitamin C, E and phenolics in *Brassica* vegetables. *J. Food Compos. Anal.* **2007**, *20*, 106–112.

(29) Lee, S. K.; Kader, A. A. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol.* **2000**, *20*, 207–220.

(30) Foyer, C. H. Ascorbic acid. In *Antioxidants in higher plants*; Alscher, R. G., Hess, J. L., Eds.; CRC Press: Boca Raton, FL, 1993; pp 32–57.

(31) Heimler, D.; Vignolini, P.; Dini, M. G.; Vincieri, F. F.; Romani, A. Antiradical activity and polyphenol composition of local *Brassicaceae* edible varieties. *Food Chem.* **2006**, . *99*, 464–469.

(32) Lako, J.; Craige Trenerry, V.; Wahlqvist, M.; Wattanapenpaiboon, N.; Sotheeswaran, S.; Premier, R. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other ready available foods. *Food Chem.* **2007**, *101*, 1727–1741.

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