Food Sci. Technol. Res., **5** (2), 125-128, 1999

Note

Investigation and Identification of Antioxidative Flavonols, Quercetin and Kaempferol Glycosides in the Unused Parts of 10 Types of Food Plants Commonly Consumed in Japan

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Received June 1, 1998; Accepted November, 7, 1998

The antioxidative flavonols,quercetin and kaempferol in the unused parts of 10 types of food plants commonly consumed in Japan were determined by paper chromatography and ultraviolet spectroscopy. The purpose was to determine a whether these portions might be usable as an antioxidant to protect foodstuffs from oxidation. The leaves of burdock and moroheiya and used tea leaves were found to contain an abundance of quercetin glycosides. These were isolated and identified as quercetin 3-*O*-ramnoglucoside (substance 1 (Tables 1, 2)) (burdock), quercetin 3-*O*-ramnoside (substance 5) (moroheiya), and quercetin 3-*O*-glucoside (substance 4) (used tea leaves). The leaves of persimmon and peach trees contained a large amount of kaempferol glycosides,and these were isolated and identified as kaempferol 3-*O*-glucoside (substance 2 (Tables 1, 2)) (persimmon), and kaempferol 3,7-*O*-glycoside- (substance 3) (peach tree). Among the flavonols, quercetin 3-*O*-glucoside (substance 1) from the leaves of burdock, and kaempferol 3,7-*O*-glycoside (substances 3 and 10)from the leaves of peach tree and ume (Japanese apricot) tree are the first reported here. Our data will provide a basis for the possible utilization of the leaves of burdock, persimmon and peach trees, and used tea leaves as antioxidants to protect foodstuffs from oxidation.

Keywords: quercetin and kaempferol, natural antioxidative flavonols, unused part of food plant

Food plant-derived flavonoids such as the flavonols quercetin, kaempferol, and myricetin have been reported to have multiple biological effects, including antioxidant activity (Larson, 1988; Takahama, 1985; Torel *et al.*, 1986; Das & Ray, 1988) and antimutagenic and anticarcinogenic effects. (Deschner *et al.*, 1991; Francis *et al.*, 1989; Fujiki *et al.*, 1986)

Antioxidative flavonols in food plants have been reported by many researchers,and in recent years Tsushida *et al.* (1994) and Tateyama *et al.* (1997) reported them in the edible parts of food plants.Little is known,however, about antioxidative flavonols in the unused portions of food plants.

We investigated antioxidative flavonols in the unused parts of 10 food plants commonly consumed in Japan to determine their possible utilization as antioxidants to protect foodstuffs from oxidation.

Emphasis was placed on the identification of the antioxidative flavonols quercetin and kaempferol, and locating unused parts of food plants which contain a large amount of these substances.

Materials and Methods

Isolation of flavonols by paper chromatography Fresh food plants (Table 1) were collected on farms or purchased in supermarkets in Yamanashi prefecture, Japan. One hundred and fifty grams of the unused part of each fresh plant was cut into small pieces, and extracted with 300 ml of 80% methanol for 7 days keeping it at 4°C refrigerator. Two milliliter of the extract was then spotted on the lower right-hand corner of a sheet of Whatman 3MM chromatographic paper (46×47 cm). A hair drier was used for solvent evaporation between repeated applications of the extracted solution to the paper. The final spot was about 3 cm in diameter.

The spotted extract on chromatographic paper was developed in a chromatocab by descending two-dimensional chromatography using tertiary butanol: glacial acetic acid: water=3:1:1 (v/v) as the first solvent, and glacial acetic acid: water=15:85 (v/v) as the second solvent.

The developed chromatogram, after drying, was viewed under a UV light in the presence of ammonia fumes. In this way, the spotted extracts on 12 sheets of chromatographic paper for each food plant were developed. Each developed chromatogram cut out from the 12 sheets of paper was extracted with 30 ml of spectroscopic methanol, and the resultant solution was stocked as the initial solution in a refrigerator and used for spectral analyses. Purity of the substance in the initial solution was confirmed by thin layer chromatography with different solvent systems.

Identification of flavonol glycoside by ultraviolet spectral analyses The concentration was first adjusted by dilution of the initial stock solution of flavonoid with spectroscopic methanol so that the optical density of the major absorption peak between 250 nm (Band II peak region) and 400 nm (Band I peak region) gave an optical density reading in the region 0.6 to 0.8. The relative content of flavonoid in the initial stock solution of each plant was

Table 1. Substances identified (or presumed) in the unused parts of 10 food plants.

Unused part of food plant (species name) (scientific name)	Place and date food plant was collected	Symbol of substance isolated from unused part of food plant	Approximate relative content of substance in the initial flavonoid stock solution (number of times diluted to get an optimum UV absorption peak with an optical density region 0.6 to 0.8)	Products identified by acid hydrolysis of substance
Leaves of burdock (Takinogawa) (*Arctium lappa* L)	Yamanashi pref, Tatomi town Nov, 1996	Substance 1	18	Quercetin Rhamnose Glucose
Leaves of persimmon tree (Kousyuu hyakume) (*Diospyros kaki* THUNB)	Yamanashi pref, Kofu city Jun, 1996	Substance 2	9	Kaempferol Glucose
Leaves of peach tree (Yamane hakutou) (*Prunus persica* BATCH *var*)	Yamanashi pref, Itimiya town May, 1997	Substance 3	14	Kaempferol Glucose
used tea leaves (Kousyuu kaiji) (*Thea sinensis* L)	Yamanashi pref, Nanbu town May, 1997	Substance 4	9	Quercetin
Leaves and stems of moroheiya (Shimathunaso) (*Corchorus olitorius* L)	Yamanashi pref, Tsuru city Jun, 1996	Substance 5	6	Quercetin Rhamnose
Leaves of radish (Aokubi) (*Raphanus sativus* L. *var. longipinnatus Bailey*)	Yamanashi pref, Tsuru city Jun, 1997	Substance 6	4	Quercetin
Leaves of carrot (Kuroda gosun) (*Daucus carota* L.)	Yamanashi pref, Tsuru city Jul, 1995	Substance 7	4	Quercetin
Leaves of sweet potato (Beniazuma) (*Ipomoea batatas* POIR.)	Yamanashi pref, Tsuru city Aug, 1995	Substance 8	2	Not identified
Leaves of celery (Konel) (Apium graveolens L var)	Yamanashi pref, Isawa town Jun, 1996	Substance 9	7	Apigenin
Leaves of ume (Japanese apricot) tree (Kousyuu koume) (*Prunus mume* SIEB)	Yamanashi pref, Isawa town Jun, 1997	Substance 10	2	Kaempferol

measured by the relative optical density (relative intensity) of the absorption peaks observed for a given flavonoid substance. The approximate contents of flavonoid in the initial stock solutions are shown in Table 1, where the numbers 1,2, 3..... represent the number of times of dilution of the initial flavonoid stock solution, to get an optimum absorption peak with an optical density reading in the region 0.6 to 0.8.

For the identification of flavonoid, the UV spectra were measured in methanol, sodium methylate, methanolic aluminum chloride, methanolic aluminum chloride-hydrochloric acid, methanolic sodium acetate, and methanolic sodium acetate-boric acid solution by the method of Mabry *et al.* (1970)

To obtain the best spectral results, a reference solution was prepared by extracting a piece of blank chromatographic paper from the same chromatogram (equal in size to the piece which contained the flavonoid) using the same procedure.

Acid hydrolysis of a glycoside to determine aglycone and sugar A substance suspected of being a glycoside was subjected to acid hydrolysis.

The flavonoid initial stock solution was concentrated almost to dryness and was dissolved in 6% aqueous hydrochloric acid (5 ml) using a minimum of methanol.

The solution was heated in a steam bath for 2 h and then cooled and extracted with ether. Evaporation of the aqueous layer yielded sugars which were identified by paper chromatography in two solvent systems: *n*-butanol: acetic acid: water= 4:1:5 v/v, and ethyl acetate: pyridine: water=2:1:2 v/v. Authentic samples of sugar were chromatographed alongside as a check. The ether layer, after drying over sodium sulfate yielded aglycone which was identified by ultraviolet spectroscopy by the six diagnostic reagents described above. All spectra were measured in a Hitachi Model 100-50 spectrophotometer.

Results and Discussion

Quercetin 3-O-rhamnoglucoside (substance 1 (Tables 1, 2)) in the leaves of a burdock (Arctium lappa L)

Purity of substance 1 was confirmed by thin layer chromatography. Substance 1 gave one spot developed chromatogram on silica gel G TLC plates with a solvent system, ethyl acetate : methyl ethyl ketone : formic acid : water (5:3:1:1), and on polyamide TLC plates, chloroform : methanol : butan-2-one (12:2:1).

The ultraviolet spectrum of substance 1 in methanol showed a peak at 257 nm and a discernible shoulder at 264 nm. (Table 2) This feature of the spectrum suggested that the substance is a flavone or flavonol which has hydroxy-substituents in both the 3′- and 4′-positions, as in luteolin or quercetin. (Fig. 1) (Mabry *et al.*, 1970) Since the addition of sodium methoxide to the substance in methanol did not induce immediate disappearance of Band I, the absence of free hydroxy-groups at both the 3-position in the C-ring and the 4′-position in the B-ring was indicated. (Mabry *et al.*, 1970)

Table 2. UV spectral data (λmax nm) of the substances isolated from the unused parts of 10 food plants.

Substance isolated from unused part of food plant	(λmax nm)						Identification (presumption) of substance isolated from unused part of food plant
	MeOH	NaOMe	$AlCl_3$	$AlCl_3$+HCl	NaOAc	NaOAc+H_3BO_3	
Substance 1	257	275	274	269	273	264	
(burdock)	264sh			297			Quercetin 3-*O*-rhamnoglucoside=
	358	423	432	358	410	384	rutin
				398			
Substance 2	265	273	276	276	272	266	
(persimmon)	298sh		305	305	306sh	302	Kaempferol 3-*O*-glucoside=astragalin
	352	324	350	350	384	362	
		397	398	398			
Substance 3	266	269	276	276	267	268	
(peach)	300sh	350sh	300	300sh	318sh		(Kaempferol 3,7-*O*-glycoside)
	349	390	357	350	356	350	
			400		400		
Substance 4	258	272	276	270	275	266	
(used tea leaves)	270sh	330	308	300	325	300sh	(Quercetin 3-*O*-glycoside)
	358	408	330	368	380	376	
			430	403			
Substance 5	256	271	274	270	268	261	
(moroheiya)	266sh	324sh	303	300	326sh	296sh	Quercetin 3-*O*-rhamnoside=
	353	396	338	356	373	370	Quercitrin
			432	403			
Substance 6	257	272	274	271	273	262	
(radish)	265sh	328sh	304	303	322sh	300sh	(Quercetin 3-*O*-glycoside)
	350	395	335	350	374	369	
			430	406			
Substance 7	257	271	274	271	261	260	
(carrot)	270sh		300	300			(Quercetin 3,7-*O*-glycoside)
	356	398	344	365	372	378	
			435	402	415sh		
Substance 8	286	272	284	286	286	288	
(sweet potato)	325	375	325	322	332	341	(4′-hydroxyflavone)
Substance 9	270	272	277	277	270	270	
(celery)	334	300sh	298	298	296sh	342	Apigenin 7-*O*-glycoside
		386	345	342			
			382	382	386		
Substance 10	266	270	275	275	266	266	
(ume)		300sh	303	300	320sh		(Kaempferol 3,7-*O*-glycoside)
	354	390	355	350	360	354	
			400	400			

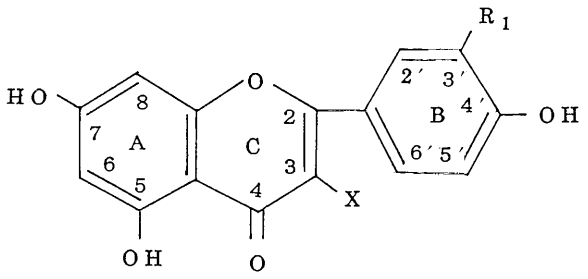

Fig. 1. Structure of flavonoids. Flavonol: X=OH, Quercetin: X=OH, R_1= OH, Kaempferol: X=OH, R_1=H. Flavone: X=H, Apigenin: X=H, R_1=H.

A bathochromic shift of 65 nm of Band I at 358 nm upon addition of sodium methoxide indicated the presence of a free hydroxy-group at the 4′-position in the B-ring. (Mabry *et al.*, 1970) Addition of anhydrous sodium acetate produced a bathochromic shift of 16 nm of Band II at 257 nm, indicating the presence of a free hydroxy-group at the 7-position in the A-ring. (Mabry *et al.*, 1970) A bathochromic shift of 26 nm of Band I upon addition of boric acid-sodium acetate suggested the presence of a free *O*-dihydroxy-grouping in the B-ring. (Mabry *et al.*, 1970) A bathochromic shift of 40 nm of Band I (in MeOH) to Band I a (in $AlCl_3$/HCl) indicated that the substance was 5-hydroxy-3-substituted flavonol. (Mabry *et al.*, 1970)

On the basis of the ultraviolet spectral characteristics described above, substance 1 was presumed to be quercetin 3-*O*-glycoside.

For the identification of glycoside, substance 1 was subjected to acid hydrolysis by the procedure described above to yield aglycone and sugar which were analyzed by paper chromatography and ultraviolet spectroscopy.

Ultraviolet spectra in six diagnostic reagents of the aglycone agreed with those of authentic quercetin and those reported for quercetin. (Mabry *et al.*, 1970) Paper chromatographic data in the two solvent systems described above agreed with those of authentic glucose and rhamnose. As a result, substance 1 was identified as quercetin 3-*O*-rhamnosyl-glucoside.

Correctness of the structure of substance 1 was confirmed by agreement of the UV spectra in six diagnostic reagents, and the paper chromatographic data (R_f value : 0.44 (tertiary

butanol : acetic acid : water=3:1:1), 0.56 (acetic acid : water=15:85)) with those of authentic quercetin 3-*O*-rhamnosyl-glucoside.

Kaempferol 3-O-glucoside (substance 2 (Table 1, 2)) in leaves of a Japanese persimmon tree (Diospyros kaki THUNB) Purity of substance 2 was confirmed by TLC in the same way as substance 1.

The ultraviolet spectrum of substance 2 in methanol showed only one peak at 265 nm in the region of Band II. (Table 2) This feature of the spectrum suggested that the substance is the 4′-oxygenated flavone or flavonol (Mabry *et al.*, 1970). Since the addition of sodium methoxide to the substance in methanol did not induce disappearance within ten min, the absence of free hydroxy-groups at both the 3-position in the C-ring and the 4′-position in the B-ring was indicated (Mabry *et al.*, 1970). A bathochromic shift of 45 nm of Band I at 352 nm upon addition of sodium methoxide indicated the presence of a free hydroxy-group at the 4′-position in the B-ring (Mabry *et al.*, 1970). Addition of anhydrous sodium acetate produced a bathochromic shift of 7 nm of Band II at 265 nm, indicating the presence of a free hydroxy-group at the 7-position in the A-ring (Mabry *et al.*, 1970). A bathochromic shift of 10 nm of Band I upon addition of boric acid-sodium acetate suggested the absence of *O*- dihydroxy-grouping in the B-ring (Mabry *et al.*, 1970). A bathochromic shift of 46 nm of Band I (in MeOH) to Band I a (in $AlCl_3$/HCl) indicated that the substance was 5-hydroxy-3-substituted flavonol (Mabry *et al.*, 1970).

On the basis of the ultraviolet spectral characteristics described above, substance 2 was presumed to be kaempferol 3-*O*-glycoside.

For the identification of glycoside, substance 2 was subjected to acid hydrolysis to yield aglycone and sugar which were analyzed by paper chromatography and ultraviolet spectroscopy.

Ultraviolet spectra in six diagnostic reagents of the aglycone agreed with those reported for kaempferol (Mabry *et al.*, 1970). Paper chromatographic data agreed with those of authentic glucose.

Substance 2 was thus identified as kaempferol 3-*O*-glucoside. Correctness of the structure of substance 2 was confirmed by agreement of the UV spectra in six diagnostic reagents, and the paper chromatographic data (R_f value: 0.70 (tertiary butanol : acetic acid : water=4:1:5) with those of authentic kaempferol 3-*O*-glucoside.

The flavonoid stock solution of substances 3, 4, 5, 6, 7, 8, 9, and 10 from the leaves of other plants shown in Tables 1, 2, were analyzed by the same procedures as those for substances 1 and 2 from the leaves of burdock and persimmon. The obtained results are shown in Tables 1 and 2.

In this work, the presence of quercetin 3-*O*-rhamnoglucoside (substance 1) from the leaves of burdock, and kaempferol 3,7-*O*-glycoside (substance 3 and 10) in the leaves of peach tree and ume (Japanese apricot) tree respectively are being reported for the first time.

We found that a large amount of quercetin was contained in the leaves of burdock, used tea leaves, and the leaves and stems of moroheiya, and a large amount of kaempferol unclerstood in the leaves of persimmon and peach trees.

A small amount of quercetin was found in the leaves of radish and carrot, and kaempferol in the leaves of ume tree.

In this study, we concentrated on the identification of the flavonol having the highest content, quercetin or kaempferol in each plant. Consequently, our results were somewhat different from those reported by other workers, whose work was on kaempferol 3-*O*-glucoside, quercetin 3-*O*-glucoside from the leaves of persimmon (Kameda *et al.*, 1987); quercetin 3-glucoside, quercetin 3-diglycoside, kaempferol diglycoside, from the leaves of small radish (Eloesser & Herrmann, 1975); luteolin 7-glucoside, apige-nin 7-glucoside from the leaves of carrot (Teubert *et al.*, 1977); and apigenin 7-glucoside, luteolin 7-glucoside, chrysoeriol 7-gluco-side, apiin from celery leaves (Galensa & Herrmann, 1980).

The data of our present study should provide a basis for the possible utilization of the unused parts of food plants such as the leaves of burdock, persimmon and peach trees, and used tea leaves as antioxidants to protect foodstuffs from oxidation.

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