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Distribution of flavonoids and related compounds from seaweeds in Japan

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Abstract: Flavonoids and related compounds were known to have the activity to affect lipid oxidation, DNA oxidation, and so on. There are many reports available to tell us the content of flavonoids in vegetables and fruits; however the content of flavonoids in seaweed is not yet available. In this study, the total amounts of rutin, quercitrin, hesperidin, myricetin, morin, luteolin, quercetin, apigenin, kaempferol, baicalein, caffeic acid and catechol in 27 Japanese seaweeds were analyzed by HPLC after hydrolysis. They were separated by an ODS-column and identified with diode array spectra. Quercetin, apigenin, kaempferol, and baicalein were not detected in all seaweeds. Hesperidin was found in all of red algae, at the amount 626-119000 μg/g dry matter. Catechol was detected all of red and green algae at the amount 1660-77700 μg/g dry matter, and the highest amount was found in *Caulerpa serrulata*. Morin was detected from all samples, and the highest amount was 3730 μg/g dry matter in *Caulerpa serrulata*. Quercitrin and myricetin were determined only from 2 seaweeds, at the amount of 202-466, and 270-346 μg/g dry matter, respectively. In general, red algae had larger amount of these compounds analyzed in this experiment than brown and green algae.

Key words: *Flavonoid contents, Japanese seaweeds, HPLC-diode array analysis*

Introduction

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4,000 different flavonoids have been described. The basic chemical structure of flavonoid is shown in Fig. 1. Since flavonoids have several hydroxyl(OH) bases in the outside of benzen rings, they were expected to express radical scavenging effect[1,2] or sometimes to have prooxidant effect as a source of reactive oxygen species.[3,4] They may have beneficial health effects because of their antioxidant properties and their inhibitory role in various stages of tumour development *in vivo*. An estimation of the total flavonoid intake to human beings is difficult, because only limited data on food contents especially from vegetables, wine, tea, fruits, etc.[5-8] are available. Recently some research made clear the loss of flavonoids during food processing[9,10] and estimated the amount of real absorption from intake.[11] However, there was no information explained about flavonoid distribution in seaweeds. We have reported catechin distribution in seaweeds in Japan, and we detected some remarkable content of catechins in the previous paper.[12] In this study, we tried to have data of flavonoid distribution in Japanese seaweed by using HPLC and photodiode array detector.

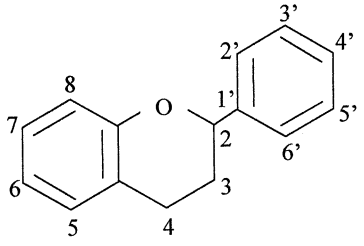

Figure 1. Basic structure of flavonoids

Materials and Methods

Materials

Green, brown, and red algae shown in Table 1 were used in this study. All samples were collected at each collection site indicated in Table 1 and the harvested samples were treated as shown in the previous report.[12]

Chemicals

Authentic rutin, caffeic acid, catechol, hesperidin, quercitrin, myricetin, morin, luteolin, quercetin, apigenin, kaempferol, and baicalein were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were commercially available.

Extraction and Hydrolysis of Flavonoids

Total flavonoids were extracted according to the method

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Table 1. Distribution of flavonoids and related compounds on seaweeds

(mean ± SD μg/g dry weight)

Scientific name (Japanese name)	Collection Site	Rutin	Caffeic acid	Catechol	Hesperidin	Quercitrin	Myricetin	Morin
Green algae								
Acetabularia ryukyuensis (Kasanori)	Ishigaki, Okinawa Pref.	26900 ± 830	317 ± 4.8	3660 ± 190	117000 ± 3300	-	-	786 ± 18
Monostroma nitidum (Hitoegusa)	Ishigaki, Okinawa Pref.	2700 ± 180	-	10200 ± 810	7500 ± 250	-	-	1320 ± 38
Tydemaniz expeditionis (Suzukakemo)	Ishigaki, Okinawa Pref.	-	-	5670 ± 2500	+	-	-	+
Caulerpa serrulata (Yorezuta)	Ishigaki, Okinawa Pref.	3370 ± 140	-	77700 ± 9300	5880 ± 570	-	-	3730 ± 23
Caulerpa racemosa (Sennarizuta)	Ishigaki, Okinawa Pref.	-	-	22000 ± 3200	1770 ± 96	-	-	1520 ± 22
Valonia macrophysa (TamagoValonia)	Ishigaki, Okinawa Pref.	-	-	4580 ± 530	2200 ± 170	-	-	1060 ± 18
Brown algae								
Undaria pinnatifida (Wakame)	Chikura, Chiba Pref.	457 ± 6.3	53.6 ± 50	1830 ± 290	-	202 ± 26	-	1020 ± 110
Eisenia bicyclis (Arame)	Chikura, Chiba Pref.	-	-	-	6930 ± 430	-	-	1860 ± 130
Hizikia fusiformis (Hijiki)	Chikura, Chiba Pref.	-	-	749 ± 540	-	-	-	1010 ± 11
Ecklonia cava (Kajime)	Chikura, Chiba Pref.	2730 ± 190	-	632 ± 140	4240 ± 380	-	-	2360 ± 280
Ishige okamurae (Ishige)	Chikura, Chiba Pref.	996 ± 48	149 ± 9.3	1660 ± 61	662 ± 49	-	-	2470 ± 300
Padina arborescens (Umiuchiwa)	Chikura, Chiba Pref.	-	-	608 ± 380	-	466 ± 640	-	1860 ± 220
Padina minor (Usuyukiuchiwa)	Ishigaki, Okinawa Pref.	-	-	-	+	-	-	767 ± 7.8
Hydroclathrus clathratus (Kagomenori)	Ishigaki, Okinawa Pref.	-	-	327 ± 49	58.3 ± 0.8	-	-	776 ± 5.7
Sargassum muticum (Tamahahakimoku)	Chikura, Chiba Pref.	-	-	+	+	-	-	927 ± 30
Tubinaria ornata (Rappamoku)	Ishigaki, Okinawa Pref.	-	-	+	+	-	346 ± 3.4	740 ± 11
Laminaria religiosa (Hosomekombu)	Kamaishi, Iwate Pref.	-	-	241 ± 33	-	-	-	1470 ± 120
Red algae								
Porphyra yezoensis (Susabinori)	Futtsu, Chiba Pref.	11400 ± 790	46.8 ± 3.8	8000 ± 4100	51300 ± 13000	-	-	771 ± 51
Chondrus verruscosus (Ibotsunomata)	Chikura, Chiba Pref.	-	74.8 ± 6.5	1660 ± 55.5	97800 ± 1780	-	270 ± 2.6	463 ± 4.3
Gelidium elegans (Makusa)	Chikura, Chiba Pref.	23200 ± 3100	132 ± 8.9	4200 ± 980	88000 ± 1300	-	-	562 ± 3.5
Chondrococcus hornemannii (Hosoba-naminohana)	Ishigaki, Okinawa Pref.	+	-	3110 ± 360	8810 ± 750	-	-	773 ± 13
Actinotrichia fragilis (Sodegarami)	Ishigaki, Okinawa Pref.	-	-	3030 ± 910	626 ± 120	-	-	257 ± 2.5
Ceratodictyon spongiosum (Kaimensou)	Ishigaki, Okinawa Pref.	4000 ± 200	-	2020 ± 260	18500 ± 2300	-	-	876 ± 17
Gracilaria texorii (Kabanori)	Chikura, Chiba Pref.	30000 ± 2900	168 ± 14	3620 ± 880	119000 ± 1800	-	-	658 ± 5.2
Gracilaria asiatica (Ogonori)	Shimonoseki, Yamaguchi Pref.	20500 ± 1900	145 ± 12	4310 ± 120	112000 ± 350	-	-	890 ± 31
Grateloupia sparsa (Hijirimen)	Chikura, Chiba Pref.	13000 ± 1700	72.9 ± 5.0	2860 ± 180	64100 ± 8600	-	-	1570 ± 54
Chondrus ocellatus (Tsunomata)	Chikura, Chiba Pref.	8830 ± 350	60.3 ± 2.6	2420 ± 150	65000 ± 1200	-	-	540 ± 4.9

+ : trace amount , − : not detected .

of Hertog *et al*.[13] as follows. Each minced fresh seaweed sample (5g) was homogenized with 40 ml of 75 % methanol with 2g /L TBHQ (t-butylhydroquinone) using a mixer (Ultra-Turrax T-25 Janke & Kunkel, GmbH Co. Staufen, Germany) at 5000-10000 rpm for 60 seconds. Ten milliliters of 6M hydrochloric acid were added and carefully mixed. The homogenate was refluxed at 90℃ for 2 hours, and final concentration of hydrochloric acid was approximately 1.2 N. After cooling, the supernatant was filtered through Advantec filter paper No. 101 (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and transferred to the volumetric flask with methanol. After replacing air with nitrogen gas in order not to decompose flavonoids, the extracts were kept at -80℃ until analysis. The effect of hydrolysis on seaweed samples by methanol-HCl mentioned above was compared with that of methanol extract. All samples were extracted and analyzed in triplicate.

Analysis of Flavonoids and Related Compounds

Flavonoids were determined by high-performance liquid chromatography modified from the methods of Hertog *et al*.[13] and Vinson *et al*.[14] Flavonoids were separated by a C_{18} column (Nova-Pak C_{18}, 4 μm, 150×3.9 mm ID, Waters Co., Milford, MA, USA) fitted with a guard column (20×3.9 mm ID), using 25 % acetonitrile in 0.025M KH_2PO_4 at pH 2.4 as mobile phase, flow rate at 0.9 mL/min and analyzed by a diode array detector SPD-M10Avp (Shimadzu Co., Kyoto, Japan). Authentic reagents were dissolved in methanol. Flavonoid concentration in the seaweed was calculated using a calibration curve within concentration 0-200 μg/mL. Samples were separated and identified by retention time and spectra of each peak.

Results and Discussion

A chromatogram of standard solution (8 flavonoids) is shown in Fig. 2. A peak of each flavonoid was identified by retention time and UV spectra. Since hesperidin and quercitrin had same retention time as shown in Fig. 3A-C, they were analyzed separately to make a calibration curve. They were identified by the difference of UV spectra as shown in Fig. 3a-b. Figure 4A shows the identification of rutin, catechol, hesperidin and morin from *Monostroma nitidum* (Hitoegusa) by a diode array detector. As shown in Fig. 3b, quercitrin had a peak at 350nm, but there was no peak at 350 nm on Fig. 4A at the same retention time. This peak had the same spectra to hesperidin as shown in Fig. 3a, therefore that peak was identified as hesperidin. There was no sample which had both quercitrin and

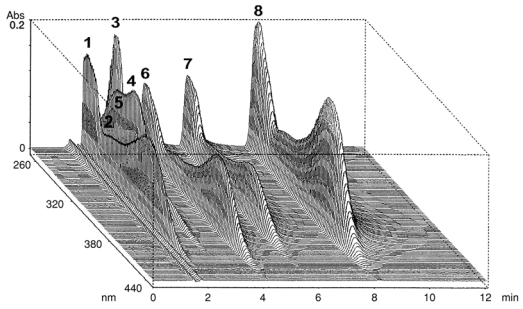

Figure 2. Photodiode array chromatogram of standards. Each peak is identified as 1: Rutin, 2: Caffeic acid, 3: Catechol, 4: Hesperidin, 5: Quercitrin, 6: Myricetin, 7: Morin, and 8: Quercetin

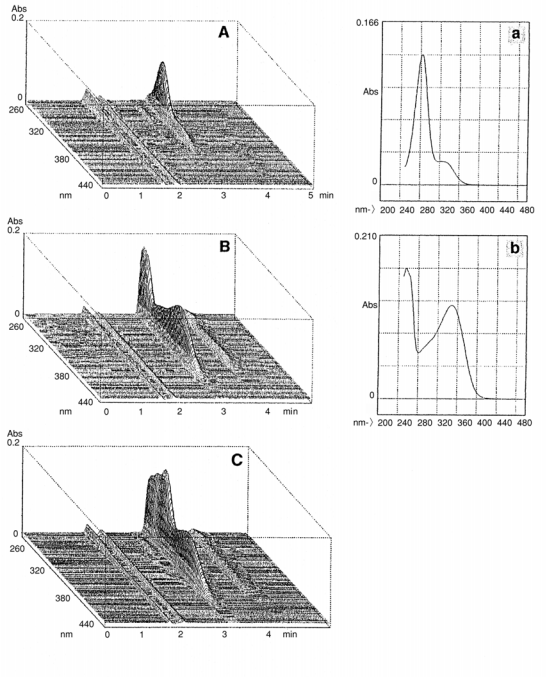

Figure 3. Identification of quercitrin and hesperidin.
A:Photodiode array chromatogram of hesperidin, a: spectra of hesperidin peak.
B:Photodiode array chromatogram of quercitrin, b: spectra of quercitrin peak.
C:Photodiode array chromatogram of hesperidin/ quercitrin mixture.

hesperidin. Effective extraction of flavonoids and related compounds was reported by Hertog *et al.*[13] Figure 4B shows the chromatogram of methanol extract (without HCl) of *Monostroma nitidum* (Hitoegusa), and there were

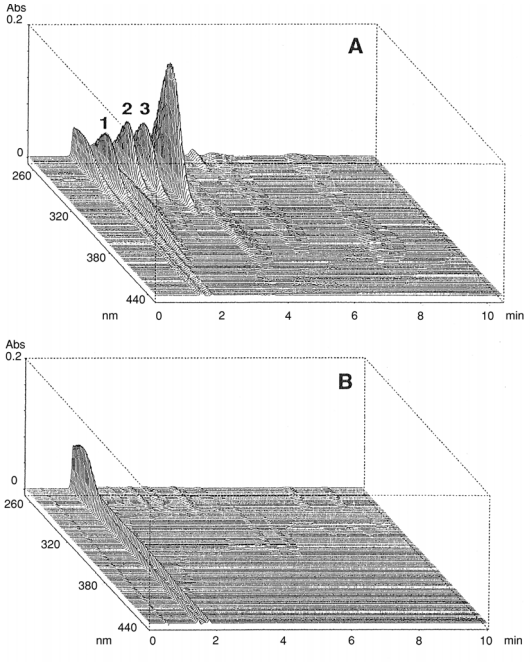

Figure 4. Photodiode array chromatogram of *Monostroma nidium* (Hitoegusa) extracts.
A: Extracted with HCl-methanol
B: Extracted with methanol
1: Rutin, 2: Catechol, 3: Hesperidin

very little peaks of flavonoids. As shown in the difference between Fig. 4A and 4B, acid hydrolysis was effective to extract total flavonoids and related compounds. Rutin includes rutinose in that chemical structure, but it was not separated by our acid extraction. We could not find rutin peak by methanol extraction, but there was a peak by methanol-acid extraction as shown in Fig. 4.

Distribution of the flavonoids in Japanese seaweeds is shown in Table 1. The results are expressed as μg of flavonoids or related compounds per g of dry weight of seaweeds.

All of the seaweeds in this study did not contain the flavonoids which had longer retention time than morin; *i.e.* luteolin, quercetin, apigenin, kaempferol and baicalein. Rutin was detected in 8 samples among 10 red algae samples, but from brown/green algae, it was detected only in 6 samples among 17 brown and green algae samples. Caffeic acid was found mainly from red algae, but the content of caffeic acid was much smaller than that of rutin, catechol, hesperidin, and morin. Catechol was detected in 25 samples, and green algae contained larger amounts than other algae. *Caulerpa serrulata* (Yorezuta), *Caulerpa*

racemosa (Sennarizuta), and *Monostroma nitidum* (Hitoegusa) contained 77700μg, 22000μg, and 10200μg of catechol, respectively. Quercitrin was found in only two samples; *Undaria pinnatifida* (Wakame) contained 202μg, and *Padina arborescens* (Umiuchiwa) 466 μg. Hesperidin was analyzed in all of red algae, and generally red algae had larger amounts of hesperidin than brown and green algae.

Chondrus verruscosus (Ibotsunomata) and *Tubinaria ornata* (Rappamoku) contained 270μg and 346μg of myricetin, respectively. Myricetin was found only from these two samples.

Morin was analyzed from all of seaweeds in this study. Brown algae contained larger amounts of morin than red algae in general, but the contant of morin varied in green algae; there were trace amounts of morin in *Tydemaniz expeditionis* (Suzukakemo), but *Caulerpa racemosa* (Sennarizuta) contained 1520μg.

Porphyra yezoensis (Susabinori) is one of the most popular cultivated edible seaweed in Japan. It had 11400 μg of rutin, 47μg of caffeic acid, 8000μg of catechol, 51300 μg of hesperidin, and 770μg of morin. Within the red algae, *Gracilaria texorii* (Kabanori) showed the highest content of rutin and hesperidin, whose concentrations were 30000 and 119000μg, respectively.

Undaria pinnatifida (Wakame) is also popular cultivated edible brown alga in Japan. It did not contain catechins as shown in our former report;[12] however, it contained rutin, caffeic acid, catechol, quercitrin, and morin. Quercitrin was detected only from this sample and *P. arborescens.* *Eisenia bicyclis* (Arame) had larger amount of catechins than other seaweeds,[12] but contained only hesperidin and morin.

Padina arborescens (Umiuchiwa) and *Padina minor* (Usuyukiuchiwa) belong to the same species, *Padina*; the former lives in the temperate zone and the latter lives in the subtropical zone. Morin was detected in both *Padina* samples, but their contents were different. *Padina arborescens* (Umiuchiwa) had 608μg of catechol and 466 μg of quercitrin, but *Padina minor* (Usuyukiuchiwa) did not contain those compounds.

Both *Caulerpa, Caulerpa serrulata* (Yorezuta) and *Caulerpa racemosa* (Sennarizuta) did not have caffeic acid, quercitrin, and myricetin. *Caulerpa serrulata* (Yorezuta) had 3370μg of rutin, but *Caulerpa racemosa* (Sennarizuta) did not contain that. Both of them contained catechol, hesperidin, and morin, but the amount of these compounds in *Caulerpa serrulata* (Yorezuta) was more than twice of the amount in *Caulerpa racemosa* (Sennarizuta).

Flavonoids and related compounds occupied more than 10% of dry weight seaweed in *Acetabularia ryukyuensis* (Kasanori), *Chondrus verruscosus* (Ibotsunomata), *Gelidium elegans* (Makusa), *Gracilaria texorii* (Kabanori) and *Gracilaria asiatica* (Ogonori). Such a large percentage of flavonoid content was also reported from *Scutellaria baicalensis* (one of the basil),[15] which was rich in baicalin, baicalein, and wogonin. However, seaweeds were rich in mainly hesperidin, and they had different flavonoid composition. From this result, we made clear that some of seaweeds are rich source of certain flavonoids.

In this study, we have dealt with the distribution of 12 flavonoids in many kinds of green, brown and red algae in Japan. However, there is no information available on the concentrations of flavonoids in seaweeds. Some researchers have already reported the content of several kinds of flavonoids in teas, cocoa, vegetables, wine, and fruits. Quercetin was analyzed from onions (284-486 mg/kg edible fresh weight), kale (110mg/kg), broccoli (30mg/kg), French beans (32-45mg/kg), and slicing beans (28-30mg/kg), and kaempferol could be detected in kale (211mg/kg), endive (15-91mg/kg), leek (11-56mg/kg), and turnip tops (31-64mg/kg).[7] Also they contained myricetin, luteolin and apigenin. It was also reported that potentially anticarcinogenic flavonoids are quercetin, kaempferol, myricetin, apigenin, and luteolin.[4, 13] They were often detected from coffee, tea, beer, and wine.[13] From all seaweeds in this study, myricetin was detected from 2 samples, but other “anticarcinogenic” flavonoids were not detected. Taken together, seaweeds have totally different flavonoid composition from vegetables and fruits.

Vinson *et al*.[14] reported the percentage of conjugated flavonoids of vegetables. From their report, some vegetables had more than 50% of conjugated form, therefore, next research about seaweed flavonoids should be including the difference of free flavonoids and conjugated flavonoids. Since some flavonoids are reported as a beneficial factor of our health, we are now testing their effect on many situations.

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Fisheries Experiment Station for collecting samples at Chikura, Chiba Pref., and Mr. Munenaka Onodera in Iwate Fisheries Technology Center for collecting sample at Kamaishi, Iwate Pref. We also thank Dr. Jiro Tanaka and Dr. Teru Ioriya, for their help with species identification. This research was partly supported by a Grant-Aid for Encouragement of Young Scientists (No. 11760146) from the Ministry of Education, Science and Culture of Japan.

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日本産海藻のフラボノイド類及び関連化合物の含量

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フラボノイド類及びその関連化合物は，脂質やDNAの酸化に対して影響力を持つとされている。本研究では日本産海藻27種類を試料とし，フラボノイド10種および関連化合物2種を，ODSカラムとダイオードアレイ検出器を用いたHPLCによって分析した。海藻からルチン，ヘスペリジン，ケルシトリン，ミリセチン，モリン，カフェイン酸，及びカテコールが検出された。ヘスペリジンは紅藻すべてから乾物1gあたり626-119000 μg検出された。カテコールは紅藻および緑藻から1660-77700 μg検出された。モリンは試料海藻すべてに存在した。一般に紅藻は褐藻および緑藻に比べてこれらの化合物の含量が高かった。

キーワード：フラボノイド含量，日本産海藻，HPLC-ダイオードアレイ分析