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The Diversity of Isoflavone Contents and *In Vitro* Antioxidant Activities in Japanese Soybean (*Glycine max* (L.) Merr.) Cultivars

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Summary

The isoflavone contents in seeds of 217 Japanese soybean cultivars were investigated by HPLC. Total isoflavones; the sum of four chemical forms (aglycones, glucosides, malonylglucosides and acetylglucosides), varied among cultivars ranging from 142.9 mg/100g soybean seeds of Osodaizu to 1171.3 mg/100g of Hiroizumi Daizu, with an average content of 487.5 mg/100g seeds. Malonylglucosides were most abundant, while acetylglucosides and aglycones were present in only trace amounts in the soybean seeds. Total phenolic, total flavonoid and relative anthocyanin contents in the seeds were found maximum in Peking. Antioxidant activities of these 217 soybean cultivars were also determined by 3 different assays; β-carotene bleaching, DPPH radical scavenging and ferric reducing assays. Soybean seed extracts showed antioxidant properties which varied widely among cultivars and assays. The highest capacities to inhibit β-carotene bleaching, to scavenge DPPH radical and to act as reducing agent were found in seeds of Hiroshima Kuro Daizu, Hazenomi Daizu and Peking, respectively. Correlations between the antioxidant contents (isoflavones, phenolics, flavonoids and anthocyanins) and the antioxidant capacities in the 3 assays were observed. The results suggest that ferric reducing powers of soybeans analyzed in this study were contributed by the presence of anthocyanins. The antioxidant activities slightly correlated with contents and kind of isoflavones.

Key Words: soybean, isoflavones, β-carotene oxidation, DPPH scavenging activity, ferric reducing power

Introduction

Soybean (*Glycine max* (L.) Merr.) has been considered to be one of the world's most important food resources. It has been used in eastern Asia, especially in China and Japan, for many centuries as the source of plant protein and vegetable oil. Besides protein and oil, soybean also has several phytochemical substances. Isoflavones are an interesting group of phytochemicals found in soybeans and soy foods. They are a group of flavonoids and well known as phytoestrogen due to their structural similarity with mammalian estradiol. Health benefits of isoflavones have bean evaluated in hormone-dependent diseases. Dietary intake of soybean isoflavones has been reported to be linked to a decreased risk of cardiovascular disease, osteoporosis, breast and prostate cancer, and menopausal symptoms[1)].

Three isoflavones (genistein, daidzein and glycitein) are present in 4 chemical forms (aglycone, glucoside, malonylglucoside and acetylglucoside) in soybean seeds. Consequently, there are totally 12 isomers of isoflavones in soybean. An objective of this study was to determine the variation of isoflavone contents in 217 Japanese soybean cultivars.

Soy isoflavones have been reported to exhibit antioxidant properties both in vitro and in vivo. Moreover, there are several classes of other antioxidant compounds present in soybeans. Therefore, the other objective of this work was to determine the antioxidant activities of Japanese soybean cultivars in order to identify those with a higher potential to promote human health by consumption of soybeans and soy products.

Materials and Methods

1. Preparation of extracts

Soybean seeds (217 cultivars) were ground and soaked in 70% ethanol at room temperature for 24 hr in the dark with agitation. The extract was centrifuged at 3000 rpm for 10 min, filtered through 0.45 μm syringe filter and stored at -20 ℃ until used.

2. HPLC analysis of isoflavones

The HPLC analysis was performed according to Kudou *et al*.[2] using YMC-Pack ODS-AM-303 (250x4.6mm) column and an Intelligence HPLC system (Jasco) composed by an autosampler (AS-2057 Plus), pump (PU-1580), and a UV/VIS detector (UV-1570). The solvent flow rate was 1ml/min and isoflavones were detected at 260 nm. The injection volume was 10 μl. A linear gradient of mobile phase was employed: solvent A was 15% acetonitrile and solvent B was 35% acetonitrile; both contain 0.1% phosphoric acid. Twelve isoflavone standards were purchased from LC Laboratories.

3. Total phenolic, total flavonoid and relative anthocyanin contents

Concentrations of 3 classes of antioxidant compounds present in soybeans were determined by the following methods. Total phenolic content was analyzed by Folin-Ciocalteu method according to Genovese *et al*.[3] with some modifications. The absorbance was measured at 700 nm. The results are expressed as gallic acid equivalent (GAE) in mg/100g soybean seeds. Total flavonoid content was determined by aluminum chloride colorimetric method[4] with some modifications. The absorbance was read at 405 nm. The results are expressed as quercetin equivalent (QE) in mg/100g of seeds. Relative anthocyanin content was determined by measuring the absorbance at 510 nm.

4. Antioxidant activity in β-carotene linoleate model system

The antioxidant activity was determined by measuring the coupled oxidation of β-carotene and linoleic acid, as described by Siddhuraju & Becker[5] with some modifications. One ml of β-carotene solution in chloroform (2 mg/10 ml) was pipetted into a round-bottom flask, which contained linoleic acid (40 mg) and Tween 40 (200 mg). After removal of the chloroform by evaporation at 45 ℃, 100 ml of oxygenated distilled water was added to the flask with vigorous agitation. Aliquots (250 μl) of this emulsion were placed in 96-well microplate, which contained 10 μl of the extracts. Samples were read against a blank containing the emulsion minus the β-carotene immediately at 450 nm (t = 0) and then at 15-min intervals for 180 min along with heat induction at 50 ℃. Antioxidant activity (AA) was calculated as percent inhibition relative to the control, using the following equation:

$$AA = \left(\frac{C\ (Abs0\text{-}Abs180) - S\ (Abs0\text{-}Abs180)}{C\ (Abs0\text{-}Abs180)} \right) \times 100$$

Where C and S are the control and samples, respectively, and Abs0 and Abs180 are the absorbance at 450 nm at 0 and 180 min, respectively.

5. DPPH radical scavenging activity

Free radical scavenging activity was estimated as described by Othman *et al*.[6] with some modifications. An aliquot of the soybean seed extract (20 μl) was mixed with 100 mM Tris-HCl buffer, pH 7.4 (80 μl). Then 100 μM 2,2-Diphenyl-1-picryhydrazyl (DPPH) previously prepared in ethanol (100 μl) was added. The mixture was stored in the dark for 20 min at room temperature. Absorbance was read at 510 nm. The scavenging effect was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \left(1 - \frac{\text{Abs of sample at 510 nm}}{\text{Abs of control at 510 nm}} \right) \times 100$$

6. Ferric reducing power

Ferric reducing power of soybean seed extract was determined based on the conversion of ferric ion into ferrous form according to the method of Shon *et al*.[7]. The extract (200 μl) was mixed with 1% potassium ferricyanide (500 μl) and 200 mM phosphate buffer, pH 6.6 (500 μl). The mixture was incubated in water bath at 50 ℃ for 20 min. Then 10% trichloroacetic acid (500 μl) was added and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (150 μl) was mixed with distilled water (150 μl) and 0.1% ferric chloride (30 μl). The absorbance was read at 700 nm. Ferric reducing power was calculated as percent relative to the power of 0.5 mg/ml gallic acid (100% reducing power).

Results and Discussion

1. HPLC analysis of isoflavones in soybeans

The similar chromatogram's patterns were observed among all of the 217 cultivars (Fig. 1) but total isoflavone contents varied widely among cultivars from 142.9 mg (Osodaizu) to 1171.3 mg (Hiroizumi Daizu), with an average amount of 487.5 mg (Table 1). These values are similar to those found in Korean cultivars (188-949 mg/100g) reported by Lee *et al*.[8]. In this study, soybean seeds were most abundant in malonylgenistin followed by malonyldaidzin, malonylglycitin, genistin, daidzin, and glycitin in the decreasing order (Fig. 2). The malonylglucoside form comprises almost 90% of total isoflavones. The total isoflavones are found to correlate with the content of malonylglucosides (r = 0.998; n = 215). The seeds contained only trace amount of aglycone and acetylglucoside forms of isoflavones.

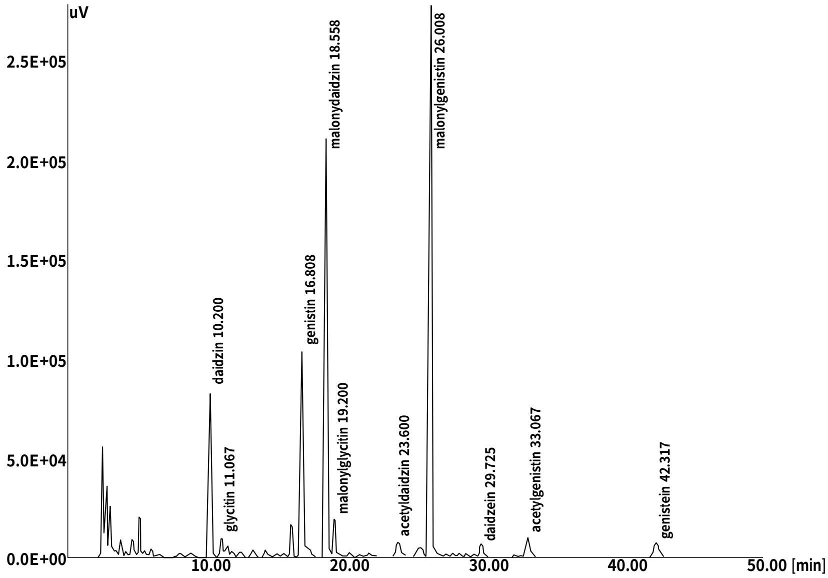

Fig. 1 Chromatogram of isoflavones extracted from soybean sample; Hiroizumi Daizu.

Table 1. Antioxidant contents and antioxidant activities in soybean extracts

No.	Soybean cultivars	Antioxidant contents				Antioxidant activities		
		Total isoflavones	Total phenolics	Total flavonoids	Anthocyanins	%AA	%SCA	%FRP
	Japanese cultivars							
1	Abura Daizu	363.1	109.64	54.59	0.005	50.11	74.83	19.07
2	Abura Mame	366.9	86.81	56.55	0.006	59.32	73.13	21.42
3	Aka Daizu (Oda Denzou)	278.9	100.47	66.06	0.030	42.19	81.03	19.91
4	Akanukanai	482.1	111.93	58.04	0.009	39.82	68.23	18.44
5	Akasaya	590.8	106.48	59.90	0.002	38.56	72.17	18.48
6	Akasaya Shouryu	739.7	142.60	69.42	0.012	32.66	77.95	20.04
7	Akatsuka	514.7	109.51	59.35	0.004	40.09	73.96	17.14
8	Akishiro	522.4	99.89	63.15	0.008	56.04	72.93	16.84
9	Akita Ani	681.8	114.10	67.42	0.008	50.43	73.72	23.31
10	Akiyoshi	680.1	96.07	57.35	0.004	52.07	66.83	15.71
11	Akiyoshi Kurakake	339.2	115.21	58.99	0.016	47.67	73.04	25.69
12	Akiyoshi Kurakake Daizu	422.7	131.26	70.75	0.025	45.15	81.42	23.43
13	Akiyoshi Shiro Daizu	674.9	122.89	65.53	0.005	64.77	79.50	19.58
14	Ao Aki Daizu	412.3	91.67	53.93	0.007	32.49	72.61	18.36
15	Ao Aki Mame	246.9	71.63	56.13	0.013	54.03	72.65	17.50
16	Ao Baka	605.7	113.64	73.95	0.012	72.25	69.44	21.42
17	Ao Ginjiro	497.4	105.28	60.15	0.014	50.55	79.20	19.65
18	Ao Mame	936.2	123.48	84.66	0.009	33.51	65.87	19.48
19	Aobata Daizu	526.6	105.54	52.93	0.016	57.14	74.00	21.28
20	Aojiro	655.3	236.98	65.55	0.008	40.59	74.13	25.07
21	Aokawa Higu	326.6	99.80	51.48	0.008	33.99	74.13	18.28
22	Aomame (Honbetsu)	266.1	98.39	38.42	0.009	39.82	72.57	14.52
23	Aoroku	460.3	110.59	59.68	0.005	48.90	74.07	16.98

24	Aoyagi	163.2	75.16	39.48	0.008	45.42	73.36	16.25
25	Asajiro 1	327.2	85.47	59.15	0.004	49.58	58.81	16.78
26	Asajiro 2	379.6	86.19	45.28	0.006	43.16	63.33	17.28
27	Ashoro	619.3	123.43	63.57	0.011	19.17	77.78	20.58
28	Ban Echigo	352.8	100.62	59.75	0.010	66.67	72.83	21.74
29	Butai Dadacha	219.6	85.45	57.48	0.019	54.36	73.72	21.76
30	Chaironodaizu	534.7	105.30	57.24	0.009	22.22	69.35	15.48
31	Chajiro	341.9	84.54	48.66	0.005	36.26	79.77	21.35
32	Chakurakake	177.6	93.97	54.46	0.018	58.61	77.08	18.34
33	Chayahori (Edamame)	406.0	105.28	59.33	0.012	51.83	80.20	24.84
34	Chikugo Daizu	363.2	101.26	48.99	0.006	35.08	77.97	20.03
35	Chizuka	353.3	103.18	54.82	0.008	62.91	70.63	20.64
36	Chougetsu	533.8	107.35	66.15	0.008	51.01	73.20	23.75
37	Choukouji Zairai	810.1	133.14	77.95	0.008	46.15	76.51	23.18
38	Choutan Daizu	499.6	102.99	60.33	0.008	52.20	67.11	16.33
39	Chuusei Date Cha	956.9	151.87	75.48	0.023	54.06	76.03	29.45
40	Daidoumame	343.7	93.76	58.77	0.006	75.21	71.66	19.90
41	Dekisugi	840.8	129.61	74.59	0.006	48.88	73.97	26.65
42	Enomoto	773.1	122.12	77.82	0.009	47.44	71.57	21.56
43	Enrei	322.2	88.46	46.48	0.003	43.47	74.50	22.01
44	ERIMO	581.7	124.89	41.30	0.012	45.64	47.79	10.28
45	Erusuta	456.8	86.61	52.55	0.009	58.53	81.44	17.69
46	Ezonishiki	225.3	75.33	51.08	0.007	67.01	74.01	20.74
47	Fujihime	584.2	119.18	64.17	0.005	39.00	75.48	17.19
48	Fukunaga 1	321.5	105.41	57.30	0.013	59.22	71.08	23.13
49	Fukuyutaka	536.0	86.28	60.88	0.007	62.06	80.00	16.73
50	Furanomaruha	447.5	95.05	55.97	0.009	32.44	73.78	16.09
51	Gankui Mame	349.3	143.92	74.06	0.074	54.64	77.78	39.51
52	Geden Shirazu	611.7	101.58	64.44	0.006	67.35	71.07	20.68
53	Gokuaomura	401.0	78.99	54.26	0.017	64.67	67.67	17.48
54	Gokuwase Chishima	269.6	131.68	56.13	0.041	49.44	75.87	28.54
55	Gokuwase Hayabusa Edamame	289.2	91.63	53.75	0.012	76.19	75.36	17.84
56	Gokuwase-kamishunbetsu	203.6	62.43	36.19	0.008	39.65	61.98	12.47
57	Goyou Daizu	378.4	100.51	56.06	0.007	53.33	77.97	24.29
58	Hachihei Mame	647.6	94.31	62.26	0.015	48.72	79.06	16.88
59	Hachikoku	228.7	68.75	49.39	0.007	39.22	54.58	11.66
60	Hakubi Daizu	276.9	100.93	42.66	0.003	35.29	73.75	20.31
61	Hana Shirazu	432.9	94.22	57.39	0.004	65.82	78.35	18.21
62	Hanayome	516.3	114.19	60.37	0.006	70.09	67.11	16.77
63	Hanguro	531.3	130.12	71.30	0.032	45.57	80.91	27.31
64	Hazenomi Daizu	437.2	123.51	67.90	0.014	45.10	81.61	26.60
65	Hidaka Aodaizu	476.5	102.56	64.39	0.017	51.28	76.35	19.46
66	Higan Mame	230.0	94.51	46.86	0.005	46.11	71.11	19.97
67	Hikage Mame	468.1	127.92	66.33	0.008	64.50	72.06	26.31
68	Hikarikuro	279.8	136.93	78.13	0.060	49.02	75.17	34.86
69	Hikuandaa	235.1	100.89	43.90	0.005	29.85	77.08	21.07
70	Hiraishi	810.9	137.47	65.75	0.005	60.62	72.86	20.79
71	Hiroizumi Daizu	1171.3	138.25	89.75	0.010	54.11	72.88	22.01
72	Hirose Kuro Daizu	461.7	158.35	88.99	0.078	50.84	77.97	42.97
73	Hiroshima Kuro Daizu	362.6	144.50	72.86	0.060	85.02	72.41	35.79
74	Hishiumi Zairai	495.9	109.86	62.75	0.008	30.92	59.97	13.48
75	Hitori Musume (Edamame)	481.1	124.88	57.99	0.011	49.15	74.51	23.72
76	Honkurakake	272.0	130.55	63.44	0.034	57.72	76.22	30.39

77	Horokanaizairai	355.1	92.55	41.59	0.008	37.14	69.79	13.13
78	Hoshi Mame	622.2	152.28	83.75	0.040	47.01	71.90	37.32
79	Hoshino Zairai	528.3	111.85	64.99	0.008	17.21	47.32	10.86
80	Hougyoku	732.0	102.28	54.90	0.006	58.22	67.84	14.24
81	Houjaku Kuwazu	586.1	106.32	67.66	0.008	9.34	45.58	11.87
82	Hyuuga	712.0	100.97	58.93	0.002	47.75	73.83	18.98
83	Ichikimame	698.4	114.00	72.24	0.009	50.73	78.21	22.50
84	Ichiryuu	420.4	128.66	62.97	0.015	62.33	72.39	23.70
85	Ichou	209.6	81.40	49.35	0.009	26.56	51.10	12.69
86	Ihhon Sangou	528.7	116.52	51.73	0.005	39.01	73.50	17.82
87	Ikki	790.9	114.11	68.55	0.013	39.45	61.40	17.31
88	Ippon Suzunari	522.1	102.64	64.44	0.007	30.77	57.86	14.63
89	Ipponsou	211.2	95.21	58.97	0.008	51.63	72.06	20.05
90	Ise Mame	431.3	96.83	59.28	0.008	27.43	51.00	12.05
91	Ishikari Shiro	418.8	103.30	64.88	0.012	21.35	74.48	20.31
92	Itachi	545.7	122.14	71.73	0.009	63.08	74.16	19.09
93	Itachikara	314.2	187.26	50.70	0.016	59.41	70.32	17.65
94	Izumi	235.5	103.15	48.66	0.016	50.26	66.83	20.21
95	Jizou	709.0	127.59	72.24	0.011	60.00	71.73	22.77
96	Kara Mame	234.9	105.82	50.19	0.009	65.27	69.44	23.79
97	Kasuga Aodaizu	335.9	95.43	64.88	0.021	38.82	73.93	16.67
98	Kawamasan (B)	283.8	91.09	46.93	0.006	76.41	70.93	19.37
99	Kemame	765.1	124.01	73.22	0.007	61.54	71.43	19.18
100	Ki Daizu (Hayashi Ayako)	386.2	101.79	61.13	0.005	28.48	81.61	21.47
101	Kimusume	334.1	94.01	61.08	0.012	60.93	71.90	19.66
102	Kin Daizu	272.2	113.65	58.84	0.008	37.73	80.06	23.75
103	Kiyomidori	531.6	100.31	62.75	0.018	36.71	75.33	23.47
104	Kizukuri Zairai	857.1	116.61	74.66	0.007	45.64	72.70	19.17
105	Kobinkatagi	465.7	98.83	66.46	0.009	56.82	74.92	18.73
106	Kosodefuri	409.8	118.85	66.57	0.013	45.97	78.13	23.64
107	Kotakeshu	418.7	86.75	55.55	0.007	22.16	52.28	12.24
108	Kou Andaa	278.6	101.60	45.99	0.005	17.21	47.89	12.09
109	Koucha	598.9	121.38	65.55	0.009	58.80	76.95	23.42
110	Kuma Daizu 1	417.2	98.39	53.77	0.005	30.50	72.99	15.99
111	Kuma Daizu 2	509.0	105.74	58.50	0.002	55.45	75.00	20.59
112	Kurokawa Seiou	304.7	126.22	61.86	0.051	56.64	74.13	30.58
113	Kurosaya Sanbongi	662.7	199.98	60.84	0.006	64.59	72.86	22.33
114	Kurumimame	547.5	99.94	65.26	0.005	55.21	72.98	14.73
115	Kyousaku Shirazu	251.3	85.17	55.75	0.007	63.42	73.86	20.34
116	Kyuushirou	681.6	125.41	76.95	0.009	63.88	66.50	20.48
117	Kyuushuu 26	725.7	116.40	66.84	0.007	34.56	80.39	27.47
118	Maedamura Zairai	448.6	136.75	76.24	0.052	66.48	79.77	36.01
119	Magarikawa Zairai	364.4	150.89	64.62	0.065	42.49	71.08	39.85
120	Mansei Ouhakushu	348.3	114.00	55.86	0.005	56.41	63.88	21.08
121	Manshuu Midori Meaka	832.0	132.60	78.15	0.013	38.13	78.30	25.92
122	Maru Shouryuu	900.1	137.35	82.26	0.012	59.48	76.56	23.22
123	Matsuzukuri	415.1	117.25	64.44	0.006	45.05	62.39	15.81
124	Menka Daizu	586.2	103.89	65.08	0.006	42.19	68.20	13.99
125	Midoridaizu	529.4	94.42	66.86	0.017	25.74	43.02	11.52
126	Miharu Daizu	408.2	104.26	54.10	0.011	33.56	71.88	16.10
127	Mikuni	307.2	85.51	57.86	0.006	24.68	50.19	10.69
128	Minoaka Daizu	360.7	83.89	57.48	0.007	48.52	71.46	13.61
129	Misaki Daizu	293.1	106.72	47.30	0.005	42.48	71.84	18.04
130	Miso Mame	400.8	100.93	59.90	0.009	46.11	73.65	21.23
131	Misuzudaizu	383.9	70.43	53.04	0.006	47.93	80.52	22.23

132	Mitama	454.8	101.26	58.86	0.011	50.09	58.41	13.11
133	Mitsu Mame	1123.1	132.65	93.19	0.009	58.46	75.04	20.54
134	Miyashiro	518.9	121.32	59.10	0.010	50.73	76.21	23.22
135	Mochi Mame	473.1	90.27	54.86	0.006	56.65	73.49	22.47
136	Mumou Hadaka	228.1	84.67	45.04	0.010	57.67	71.08	22.39
137	Murasaki No Mi	476.6	114.05	56.77	0.009	66.36	70.92	21.23
138	Murayutaka	436.0	72.12	50.82	0.005	56.99	77.78	17.26
139	Mushi Shirazu (1)	309.8	99.66	52.88	0.006	73.33	73.72	21.03
140	Nadeshiko	354.2	83.56	50.66	0.005	60.28	72.06	19.45
141	Nagon	570.9	116.52	66.15	0.013	68.37	68.46	21.26
142	Narisuke	352.6	89.15	47.42	0.010	38.61	72.36	17.53
143	Nishitsugaru Zairaishu	472.3	95.62	58.06	0.008	46.63	72.54	20.74
144	Nourin 1	804.5	128.75	68.84	0.011	53.15	76.50	28.59
145	Nukanaidaizu	574.9	134.05	75.75	0.011	23.71	76.91	21.12
146	Ogasawara Zairai	508.4	110.55	57.64	0.006	65.47	71.37	17.97
147	Oho Mame	324.2	80.16	46.15	0.012	53.30	65.81	16.06
148	Okuhara Daizu	393.1	95.35	56.19	0.010	60.35	66.49	19.13
149	Ookimame	709.3	108.45	71.64	0.008	39.03	55.94	12.34
150	Oomejiro	293.8	101.72	48.79	0.005	44.97	49.42	13.26
151	Oono Zairai	457.0	113.57	60.53	0.008	53.80	80.65	23.41
152	Ootama	627.1	100.39	67.48	0.003	56.75	66.51	18.33
153	Ootsuru	464.1	95.21	60.08	0.011	68.82	76.00	23.65
154	Ootsuura	319.0	108.25	55.64	0.011	55.44	66.03	16.86
155	Oraku Mame	518.7	111.67	59.42	0.008	54.03	76.06	21.75
156	Osodaizu	142.9	106.89	44.66	0.003	44.23	62.64	12.74
157	Otofuke Kurakake	550.7	123.01	62.97	0.008	28.86	75.76	21.67
158	Rankoshi	790.7	124.22	85.10	0.010	44.88	72.57	20.77
159	Rokugatsu Daizu	606.8	104.30	76.99	0.014	47.44	68.30	17.82
160	Ryokushoku shasei	544.3	145.74	56.88	0.008	31.26	61.11	16.40
161	Sachiyutaka	532.8	101.50	51.64	0.005	54.99	80.39	19.55
162	Sagamidori 4	593.6	93.56	53.10	0.011	45.78	78.04	17.40
163	Sagi Shiro Daizu	576.3	114.15	64.59	0.009	32.49	81.42	17.36
164	Saishuutou Shirokotsubu	372.8	78.45	56.35	0.006	24.68	37.04	10.25
165	Satou Daizu	683.1	141.05	74.68	0.009	35.04	75.00	27.38
166	Satouirazu	668.9	121.01	69.86	0.003	56.43	75.33	23.74
167	Senbirishu	271.1	102.93	45.93	0.005	48.77	66.78	15.66
168	Sennari Musume	785.6	131.54	74.46	0.004	56.07	74.45	21.81
169	Shibetsunagaha	420.6	103.51	51.15	0.014	32.44	69.27	16.26
170	Shichigatsu Mame	510.8	144.47	84.33	0.070	70.64	69.52	41.37
171	Shichigou Cha Mame	382.0	85.51	57.82	0.010	27.22	36.97	9.26
172	Shimokusano	581.1	107.64	63.13	0.009	50.00	78.49	20.64
173	Shimoshirazu	425.8	99.61	59.50	0.009	51.16	72.88	20.65
174	Shin Tamanishiki	314.6	96.94	56.39	0.010	54.40	76.21	21.38
175	Shinroku	557.3	129.90	67.99	0.011	46.98	71.73	22.77
176	Shiranui	348.0	106.73	62.86	0.007	26.94	57.30	14.14
177	Shiratama	576.9	96.98	65.68	0.005	68.21	71.43	21.26
178	Shiro Daizu	640.8	116.80	67.26	0.005	53.59	80.46	19.82
179	Shiro Gankui	512.6	129.69	67.10	0.008	63.56	74.44	24.33
180	Shiro Zairai	315.1	83.22	55.88	0.005	40.51	70.11	16.36
181	Shirohana	482.0	106.67	54.84	0.007	65.30	74.74	18.69
182	Shirohana Oosodefuri	594.0	112.51	64.10	0.010	28.98	77.95	20.05

183	Shirosaya	318.0	110.72	46.48	0.004	33.12	77.08	18.97
184	Shokuyou Aki Daizu	573.2	109.62	61.50	0.006	51.05	68.95	16.86
185	Tamawata	489.6	89.90	55.10	0.006	55.30	72.29	15.89
186	Tanbakuro	774.4	144.38	72.55	0.035	69.89	73.83	36.19
187	Tanokuromame (Edamame)	490.1	104.81	54.70	0.006	54.03	77.64	18.53
188	Tochigi Kuro Sengoku	646.3	180.56	85.97	0.086	56.99	67.32	43.85
189	Totsuta Daizu	619.8	207.14	63.70	0.013	45.97	75.87	18.78
190	Tounou Keitou	598.8	103.31	69.88	0.015	47.91	54.25	12.33
191	Toyoshirome	651.5	107.22	56.48	0.007	50.84	79.61	17.84
192	Tsurunoko Shirohana	520.3	101.47	57.06	0.002	32.89	60.96	12.78
193	Urayama Wase	663.9	117.88	74.48	0.009	45.89	74.18	23.70
194	Uzura Mame	437.1	87.88	64.35	0.016	55.56	73.33	21.70
195	Wase Bon	209.6	89.44	48.90	0.005	70.64	69.37	17.60
196	Wase Keburi	379.5	85.66	65.48	0.007	56.30	74.60	22.87
197	Wase Mame	182.5	108.47	46.68	0.012	36.02	58.04	14.83
198	Yagi	576.0	115.70	71.22	0.009	35.71	75.50	23.16
199	Yagimame	709.7	105.95	77.53	0.005	58.38	72.38	22.39
200	Yamaguchi Aki Daizu	491.7	111.14	62.17	0.005	46.84	66.86	11.12
201	Yogore Mame	454.6	104.10	56.08	0.013	57.36	59.15	16.14
202	Yoshioka Dairyuu	429.7	111.01	61.48	0.012	21.79	77.08	23.42
203	Yougetsu	629.8	112.41	67.90	0.006	51.81	72.86	23.92
204	Yukikorogashi (Kashima)	450.3	95.31	60.84	0.008	57.33	71.81	16.98
205	Yukinoshita	431.6	111.68	63.53	0.020	59.48	77.78	19.82
206	Yukiwari Mame	451.2	108.70	56.22	0.008	68.55	74.01	20.16
207	Zenkou Mame	640.6	127.35	68.35	0.009	45.27	60.13	16.21
208	Zunda Mame	868.5	117.80	68.64	0.006	61.88	74.01	19.40
	Foreign cultivars							
1	Bay	579.8	87.59	42.37	0.004	59.45	56.99	12.25
2	Clark	775.8	77.35	63.33	0.009	42.40	79.87	22.72
3	Jack	505.3	103.72	56.75	0.007	50.54	80.13	23.26
4	Peking	815.0	256.24	101.55	0.121	72.50	60.33	78.44

Total isoflavones are shown in mg/100g fresh weight; total phenolics are shown in mg GAE/100g f.w.; total flavonoids are shown in mg QE/100g f.w.; Anthocyanin contents are expressed as OD_{510} of the extracts; AA: antioxidant activity; SCA: scavenging activity; FRP: ferric reducing power.

2. Total phenolic, total flavonoid and anthocyanin contents

Phenolic compounds are very important plant metabolites because of their physiological functions against oxidative damage. Flavonoids are phenolic compounds and anthocyanins are flavonoids, which are very effective antioxidants. Total phenolic contents per 100g soybean seeds varied ranging from 62.43 mg GAE (Gokuwasekamishunbetsu) to 256.24 mg GAE (Peking). Total flavonoids in seeds were also lowest for Gokuwasekamishunbetsu (36.19 mg QE/100g) and highest for Peking (101.55 mg QE/100g). Anthocyanin contents in seeds were lowest for Tsurunoko Shirohana, Akasaya, Hyuuga and Kuma Daizu 2 and highest for Peking. The cultivars with black seed coat, especially Peking, possessed high amount of these antioxidant compounds.

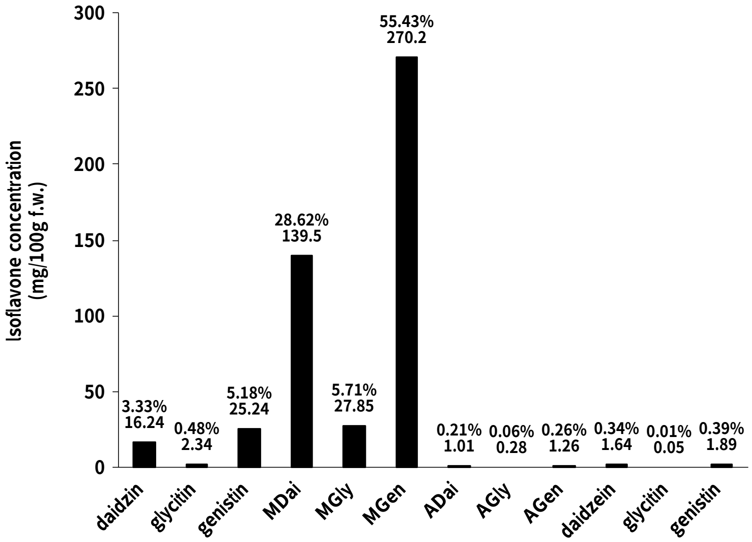

Fig. 2 Average concentrations of 12 isoflavones calculated from the contents of 217 cultivars used in this study; numbers above bars indicate relative percent to total isoflavones and the concentration (mg/100g) of each isoflavone; MDai: malonyldaidzin, MGly: malonylglycitin, MGen: malonylgenistin, ADai: acetyldaidzin, AGly: acetylglycitin, AGen: acetylgenistin.

3. Antioxidant activity in β-carotene linoleate model system

This assay was performed to determine the ability of soybean seed extracts to inhibit β-carotene oxidation by neutralizing the linoleate-free radical occurred in β-carotene linoleate model system. In the reaction containing the extracts, the degradation of β-carotene was slower and the β-carotene content remaining in the system was higher than that of control reaction after 3 hr from starting the assay (Fig. 3). Soybean extracts of all cultivars analyzed in this study showed antioxidant activity. The antioxidant activity (AA) values ranged from 9.34% in Houjaku Kuwazu to 85.02% in Hiroshima Kuro Daizu, but most cultivars exhibited moderate values between 40 and 60%. The ability to inhibit β-carotene oxidation did not correlate with total isoflavone (r = 0.033), total phenolic (r = 0.116) and total flavonoid (r = 0.133) contents. However, it showed correlation with anthocyanin contents (r = 0.217; $P < 0.05$).

4. DPPH radical scavenging activity

The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. DPPH radical is a stable proton free radical whose purple color fades rapidly when it encounters proton radical scavengers[6]. This assay determines the hydrogen-donor capability of antioxidants. The scavenging activities in cultivars tested ranged from 36.97% (Shichigou Cha Mame) to 81.61% (Hazenomi Daizu). Most cultivars showed high activity between 70 and 80%. DPPH radical scavenging activity did not correlate with total isoflavone (r = 0.126), total phenolic (r = 0.126), total flavonoid (r = 0.140) and relative anthocyanin (r = 0.047) contents.

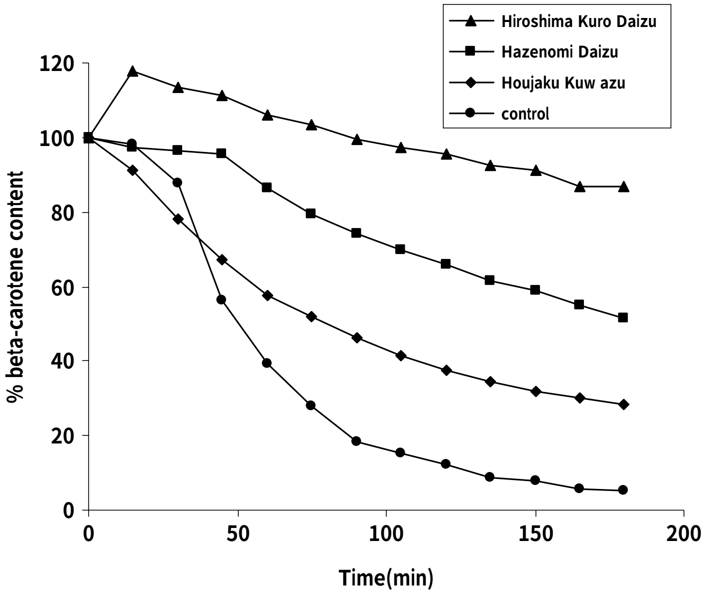

Fig. 3 Changes in β-carotene content during the incubation at 50℃ for 180 min in the cultivars with highest, medium and lowest AA (Hiroshima Kuro Daizu, Hazenomi Daizu and Houjaku Kuwazu, respectively).

5. Ferric reducing power

This assay measures the potential of antioxidants present in soybean seeds to act as reducing agent which can be monitored spectrophotometrically at 700 nm. Higher intensity of the absorbance indicates higher reducing power. Ferric reducing powers among cultivars tested ranged from 9.26% (Shichigou Cha Mame) to 78.44% (Peking). The reducing power of the soybean extracts did not correlate with total isoflavones (r = 0.170). While it positively and significantly correlated with total phenolic (r = 0.624; P<0.001), total flavonoid (r = 0.551; P<0.001) and especially with total anthocyanin (r = 0.822; P<0.001) contents. It is concluded that ferric reducing powers of soybeans tested in this study were contributed by the presence of anthocyanins.

In conclusion, there are differences in the contents of each antioxidant compounds among Japanese soybean cultivars. We consider that among 217 cultivars used in this study, the cultivars with black seed coat are recommended to serve as dietary consumption. Especially, Peking can be considered as the superior phytonutrient supplier since this cultivar has high isoflavone, phenolic, flavonoid and anthocyanin contents. Moreover, it also showed high antioxidant activities evaluated in all 3 assays.

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日本産ダイズにおけるイソフラボン含量および抗酸化活性の品種間差異について

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摘　　要

我が国原産のダイズ品種を中心として，佐賀大学が保有する217品種を供試して，その重要な機能成分であるイソフラボン含量の品種間差異を検討した．その含量は，143ｇ-1,171ｇ/100ｇと低含有から高含有のものまで，広範囲に分布した．すべての品種において，主要なイソフラボンはマロニル配糖体であった．さらに３種の測定法による抗酸化活性の測定をおこない，イソフラボン含量に加え，フェノール，フラボノイド，アントシアニン含量を定量し，これらの相関関係を検討した．抗酸化活性との関連はイソフラボンでは低く，アントシアニン含量との関連が高く，なかでも黒ダイズ品種の抗酸化活性は顕著であった．なかでも「ペキン」は抗酸化活性が高く，しかもイソフラボンも高含有しており，機能性ダイズ新品種開発の素材として注目された．