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Original article

Antiglycation effect of various vegetables: Inhibition of advanced glycation end product formation in glucose and human serum albumin reaction system

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Abstract

***Purpose*:** We investigated the antiglycation effect of various commercial vegetables comparatively in an *in vitro* glycation reaction model using various commercial vegetables in dried, powdered form.

***Method*:** We studied 187 commercial vegetables using aminoguanidine (AG) as a positive control. Using glucose/human serum albumin (HSA) as an *in vitro* glycation reaction model, 8 mg/mL HSA and 0.2 mol/L glucose were brought to reaction in a phosphate buffer solution at 60°C for 40 hours, and formation of advanced glycation end products (AGEs) was measured quantitatively with an excitation wavelength of 370 nm at a fluorescence wavelength of 440 nm. Various experimental materials were added to the model, and the 50% inhibitory concentration (IC_{50}) for AGE formation at such time was calculated.

***Results*:** The results for antiglycation activity measured for 187 vegetable samples produced IC_{50} values for inhibition of fluorescent AGE formation equivalent to or higher than that of AG (0.067-0.100 mg/mL) in the following samples: Chestnut, outer skin (*Castanea crenata*) 0.015 mg/mL; water chestnut, seed coat (*Trapa japonica*) 0.017 mg/mL; chestnut, soft layer 0.030 mg/mL; nalta jute (*Corchorus olitorius*) 0.040 mg/mL; new ginger (*Zingiber officinale*) 0.054 mg/mL; rosemary (*Rosmarinus officinalis*) 0.059 mg/mL; mugwort, powder (*Artemisia indica*) 0.070 mg/mL; lady's thumb (*Polygonum hydropiper*) 0.072 mg/mL; red leaf lettuce (*Lactuca sativa*) 0.075 mg/mL; rice eggplant, peel (*Solanum melongena*) 0.081 mg/mL. By vegetable type, IC_{50} mean values for antiglycation activity were strongest in herbs (0.230 mg/mL), leaf vegetables (0.251 mg/mL), and stem vegetables (0.321 mg/mL) in that order, and IC_{50} values in seeds, beans, and grains showed virtually no anti-glycation activity, at 10,000 mg/mL or higher. By taxonomic family, IC_{50} mean values for vegetables were highest in samples of Asteraceae (0.171 mg/mL), Lamiaceae (0.201 mg/mL), Basellaceae (0.238 mg/mL), Polygonaceae (0.278 mg/mL), and Apiaceae (0.367 mg/mL) in that order. Antiglycation activity was low in specimens of Poaceae (Gramineae), Cucurbitaceae, and Fabaceae. Results for predicted effect calculated on a reference level of the recommended daily intake of each vegetable showed that 185 of the 187 vegetable samples tested could have an antiglycation effect equivalent to or greater than that of AG.

***Conclusion*:** Vegetables in the Asteraceae, Lamiaceae, Basellaceae, and Polygonaceae families have an inhibitory effect on AGEs equivalent to or better than that of AG, and consumption of these vegetables may alleviate glycation stress.

***KEY WORDS*:** glycation stress, advanced glycation end products (AGEs), vegetables, polyphenols

Introduction

Glucose and other reducing sugars are an essential nutrient for sustenance of life. However, fructose, glucose, and other sugars bind to proteins non-enzymatically *in vivo* and alter the structure and function of proteins. This phenomenon is known as a glycation reaction and leads to formation and accumulation of advanced glycation end products (AGEs). In turn, binding of AGEs to receptors (receptors for AGEs, "RAGE") causes degradation of functional proteins and damage to tissues. Accumulation of AGEs also contributes to the onset and progression of diabetic complications, osteoporosis, and lifestyle-related diseases such as arteriosclerosis. *In vivo* glycation represents a risk factor for accelerated aging and is known as glycation stress, a topic of recent interest [1]. Mitigation of glycation stress to inhibit accumulation of AGEs is believed to play a role in prevention of lifestyle and other such diseases.

Plants contain various nutritional and functional components such as vitamins and minerals, dietary fiber, and phytochemicals and are also replete with components useful for maintenance of health. Herbs, health teas, and other plants are also known to contain functional components

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which inhibit glycation stress. However, research concerning ingredients such as fruits, vegetables, and similar items is extremely limited. To determine whether heretofore unknown functionality exists in plants which may be consumed in the form of familiar food products, we used an *in vitro* glucose/ human serum albumin (HSA) model [2] to investigate inhibitory effect on glycation reactions.

Method

Sample preparation

The samples used were 187 varieties of commercial vegetables. First, five different types of commercial vegetables (cabbage, white potatoes, daikon, bell peppers, tomatoes) were sliced thinly into 10 mm and 5 mm slices in each case and dried, and the samples were then weighed at 0, 1, 3, 5, 10, 20, and 30 hours to establish parameters. At 20 hours and 30 hours, vegetable sample weights were found to be virtually unchanged by drying. Change in sample weight was compared among the 10 mm and 5 mm slices of potatoes, daikon, and tomatoes, and the elapsed time required for weight stabilization was found to be 20 hours among the 10 mm slices and 10 hours among the 5 mm slices. On this basis, a drying time of 20 hours and a slice thickness of 5 mm or less were established as sample preparation parameters. Dried powders were produced by drying, disinfecting, and pulverizing the starting vegetable material, and then sieving (approx. 120-mesh) the material obtained. Aminoguanidine hydrochloride ("AG"; Wako Pure Chemical Industries, Chuo-ku, Osaka) was used as a positive control.

Measurement of inhibitory activity for fluorescent AGE formation

AGE-derived fluorescence was measured as reported previously using a glucose HSA model [2]. 100 μL of various concentrations of vegetable powder or AG in aqueous solution were added to 500 μL 0.1 mol/L phosphate buffer solution (PBS, pH 7.4), 100 μL distilled water, 200 μL 40 mg/mL HSA (Sigma Chemical Co., Ltd; St. Louis MO, USA), and 100 µL 2.0 mol/L aqueous solution of glucose. Distilled water was then added to make up a total volume of 1.0 mL, and the material was incubated at 60°C for 40 hours (Solution A). Final concentrations were 8 mg/mL HSA and 0.2 mol/L glucose. At the same time, a solution including distilled water added in lieu of aqueous glucose was incubated as a blank for each reaction (Solution B). Samples prepared without the addition of dried vegetable powder or AG were incubated as a positive control (Solution C). And at the same time, a solution including distilled water added in lieu of aqueous glucose was incubated as a blank for positive controls (Solution D). Fluorescent AGEs were measured quantitatively in each sample reaction solution (A, B, C, D) to evaluate inhibitory activity for AGE formation. AGE-derived fluorescence was measured using an ARVO MX 1420 ARVO series Multilabel Counter (Perkin-Elmer Japan Corp; Hodogaya-ku, Yokohama, Japan) microplate reader at an excitation wavelength of 370 nm and a fluorescence wavelength of 440 nm. Calibration curves for inhibition of fluorescent AGE formation (%) were constructed by adding individual samples to a reaction solution at three concentrations (0.1%, 0.01%, 0.001%) and calculating inhibition of AG formation (%) after the reaction. Inhibition of AG formation (%) was calculated using the following formula, and 50% inhibitory concentration (IC_{50}) values were then calculated to represent antiglycation activity.

$$\text{Inhibition of fluorescent AGE formation (\%)} = (1-(A-B)/(C-D)) \times 100$$

Reported IC_{50} values for AG are 0.063 mg/mL [3], 0.068 mg/mL [4], and 0.080 mg/mL [5].

Results

185 of the 187 vegetable samples tested demonstrated an antiglycation effect in the *in vitro* glucose HSA reaction model, *i.e.*, an inhibitory effect on formation of fluorescent AGEs (***Table 1, Fig. 1***). Antiglycation activity, in decreasing order, was as follows: Chestnut, outer skin (*Castanea crenata*) 0.015 mg/mL; water chestnut, seed coat (*Trapa japonica*) 0.017 mg/mL; chestnut, soft layer 0.030 mg/mL; nalta jute (*Corchorus olitorius*) 0.040 mg/mL; new ginger (*Zingiber officinale*) 0.054 mg/mL; rosemary (*Rosmarinus officinalis*) 0.059 mg/mL; mugwort, powder (*Artemisia indica*) 0.070 mg/mL; lady's thumb (*Polygonum hydropiper*) 0.072 mg/mL; red leaf lettuce (*Lactuca sativa*) 0.075 mg/mL; rice eggplant, peel (*Solanum melongena*) 0.081 mg/mL. Among the AG positive controls, IC_{50} values were 0.067 0.100 mg/dL. However, two varieties, Proso millet (*Panicum miliaceum*) and jelly ear (*Auricularia auricula*) showed no activity at all.

Antiglycation activity was also compared among the 185 vegetable samples classified as fruit vegetables (46), leaf vegetables (41), stem vegetables (9), root vegetables (27), seeds (15), beans (12), grains (13), mushrooms (8), and herbs (14) (***Fig. 2***). The results showed that the samples with the lowest IC_{50} values in each category were rice eggplant (peel, 0.081 mg/mL) among fruit vegetables, nalta jute (0.040 mg/mL) among leaf vegetables, red rhubarb (peel, *Rheum rhabarbatum*, 0.107 mg/mL) among stem vegetables, new ginger (0.054 mg/mL) among root vegetables, chestnut (outer skin, 0.015 mg/mL) among seeds, black soybeans (Glycine max, 0.333 mg/mL) among beans, red-kernelled rice (Oryza sativa subsp. Japonica, 0.433 mg/mL) among grains, shiitake mushrooms (pileus, *Lentinula edodes*, 0.550 mg/mL) among mushrooms, and rosemary (0.059 mg/mL) among herbs. By vegetable type, IC_{50} mean values for antiglycation activity were strongest in herbs (0.230 mg/mL), leaf vegetables (0.251 mg/mL), and stem vegetables (0.321 mg/mL) in that order, and IC_{50} values in seeds, beans, and grains showed virtually no antiglycation activity, at 10,000 mg/mL or higher.

With reference to the class-level phylogeny of angiosperms through the Angiosperm Phylogeny Website (2008) [6], and considering only families which included three or more samples of vegetables or herbs in each family, samples were classified into 18 families respectively, and antiglycation activity was compared (***Fig. 3***). By vegetable family, IC_{50} values (mean, by family) for vegetables were highest in specimens of Asteraceae (12 samples, 0.171 mg/mL), Lamiaceae (9 samples, 0.201 mg/mL), Basellaceae (3 samples, 0.238 mg/mL), Polygonaceae (4 samples, 0.278 mg/mL), and Apiaceae (11 samples, 0.367 mg/mL) in that order. Antiglycation activity was low in specimens of Poaceae (Gramineae, 11 samples), Cucurbitaceae (8 samples), and Fabaceae (17 samples), with IC_{50} values (mean) at 10,000 mg/mL or higher, and standard deviations of 10,000 mg/mL or higher. Comparison among vegetable families showed large differences in IC_{50} among various samples.

Table 1. Antiglycation activity of 187 vegetables and quantitative intake for equivalence to aminoguanidine (AG)

ID	Common Name	Japanese name	Scientific Name	Vesicaria	Family	IC_{50} (mg/mL)	Daily AG Eq (g)
1	Green spring onion	Ao-negi	*Allium fistulosum*	Leaf vegetable	Alliaceae	0.179	5.5
2	Avocado	Abogado	*Persea Americana*	Fruit vegetable	Lauraceae	0.220	2.1
3	Amanaga chili pepper	Amanaga-tougarashi	*Amanaga capsicum*	Fruit vegetable	Solanaceae	0.254	1.5
4	Edamame	Eda-mame	*Clycine max*	Bean	Fabaceae	1.187	10.8
5	Pumpkin	Kabocha	*Cucurbita*	Fruit vegetable	Cucurbitaceae	12.060	160.1
6	Sweet potato	Satsuma-imo	*Lpomoea batatas*	Root vegetables	Convolvulaceae	12.084	132.8
7	Eddoe	Sato-imo	*Colocasia esculenta*	Root vegetables	Araceae	1.677	28.9
8	Kidney bean	Saya-ingen	*Phaseolus vulgaris*	Fruit vegetable	Fabaceae	0.698	21.8
9	Potato	Jaga-imo	*Solanum tuberosum*	Root vegetables	Solanaceae	5.624	82.3
10	Jumbo kidney bean	Jumbo-ingen	*Phaseolus vulgaris*	Fruit vegetable	Fabaceae	2.216	92.3
11	Ginger	Shouga	*Zingiber officinale*	Root vegetables	Zingiberaceae	0.177	3.1
12	Welsh onion	Shiro-negi	*Allium fistulosum*	Leaf vegetable	Alliaceae	0.610	17.8
13	Zucchini	Zucchini	*Cucurbita pepo*	Fruit vegetable	Cucurbitaceae	0.568	28.4
14	Broad bean	Sora-mame	*Vicia faba*	Bean	Fabaceae	1.912	21.3
15	Daikon (Japanese radish)	Daikon	*Raphanus sativus*	Root vegetables	Vesicaria	0.637	38.2
16	Onion	Tama-negi	*Allium cepa*	Root vegetables	Amaryllidaceae	0.581	15.4
17	Winter melon	Tougan	*Benincasa hispida*	Fruit vegetable	Cucurbitaceae	1.406	100.4
18	Corn	Toumorokoshi	*Zea mays*	Grain	Poaceae (Gramineae)	4.734	55.5
19	Chinese yam	Naga-imo	*Dioscorea batatas*	Root vegetables	Dioscoreaceae	3.154	71.1
20	Chinese chive	Nira	*Allium tuberosum*	Leaf vegetable	Amaryllidaceae	0.353	14.3
21	Garlic	Ninniku	*Allium sativum*	Root vegetables	Amaryllidaceae	6.130	23.4
22	Broccoli (Stem)	Broccoli	*Brassica oleracea*	Fruit vegetable	Vesicaria	0.199	5.5
23	Broccoli (Flower bud)	Broccoli	*Brassica oleracea*	Fruit vegetable	Vesicaria	0.384	14.8
24	Lotus roots	Renkon	*Nelumbo nucifera*	Root vegetables	Nelumbonaceae	0.275	6.9
25	Okra	Okura	*Abelmoschus esculentus*	Fruit vegetable	Malvaceae	0.504	15.9
26	Carrot	Ninjin	*Daucus carota*	Root vegetables	Apiaceae	1.342	34.1
27	Cherry tomato	Mini-tomato	*Lycopersicon esculentum*	Fruit vegetable	Solanaceae	0.645	21.7
28	Tomato	Tomato	*Solanum lycopersicum*	Fruit vegetable	Solanaceae	0.632	27.5
29	Red onion	Red onion	*Allium cepa*	Root vegetables	Amaryllidaceae	0.401	12.2
30	Eggplant	Nasu	*Solanaceae*	Fruit vegetable	Solanaceae	0.228	9.9
31	Great burdock	Gobou	*Arctium lappa*	Root vegetables	Asteraceae	0.315	4.7
32	Cucumber	Kyuri	*Cucumis sativus*	Fruit vegetable	Cucurbitaceae	0.423	27.0
33	Bitter melon	Gouya	*Momordica charantia*	Fruit vegetable	Cucurbitaceae	0.685	33.1
34	Celery (Stem)	Serori	*Apium graveolens*	Stem vegetables	Apiaceae	0.403	22.0
35	Celery (Leaf)	Serori	*Apium graveolens*	Stem vegetables	Apiaceae	0.120	3.9
36	Cabbage	Kyabetsu	*Brassica oleracea*	Leaf vegetable	Vesicaria	0.313	11.9
37	Lily bulb	Yurine	*Lilium*	Root vegetables	Liliaceae	7.010	69.9
38	Shiitake mushroom (Pileus)	Shii-take	*Lentinula edodes*	Mushroom	Tricholomataceae	0.550	20.6
39	Shiitake mushroom (The foundation)	Shii-take	*Lentinula edodes*	Mushroom	Tricholomataceae	0.634	19.2
40	Hen of the woods	Mai-take	*Grifola frondosa*	Mushroom	Meripilaceae	1.876	59.9
41	King oyster	Eringi	*Pleurotus eryngii*	Mushroom	Pleurotaceae	2.438	64.7
42	Buna-shimeji	Buna-shimeji	*Hypsizygus marmoreus*	Mushroom	Lyophyllaceae	1.464	44.4
43	Pea	Kinusaya	*Pisum sativum*	Fruit vegetable	Fabaceae	0.284	9.6
44	Enoki mushroom	Enoki-dake	*Flammulina velutipes*	Mushroom	Tricholomataceae	22.016	485.6
45	Bean sprout	Midorimame-moyashi	*Vigna radiate*	Leaf vegetable	Fabaceae	0.584	48.7
46	Garlic shoots	Ninniku-no-me	*Allium sativum*	Stem vegetables	Amaryllidaceae	0.188	5.1
47	Bell pepper	Piman	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.146	6.5
48	Pak choy	Chingen-sai	*Brassica rapa*	Leaf vegetable	Vesicaria	0.185	13.5
49	Mioga ginger	Myouga	*Zingiber mioga*	Fruit vegetable	Zingiberaceae	0.198	14.5
50	Asparagus	Asuparagasu	*Asparagus officinalis*	Stem vegetables	Liliaceae	0.209	11.6
51	Broccoli sprouts	Hatsuga-broccoli	*Brassica oleracea*	Leaf vegetable	Vesicaria	0.101	7.6

ID	Common Name	Japanese name	Scientific Name	Vesicaria	Family	IC_{50} (mg/mL)	Daily AG Eq (g)
52	Broccoli super sprouts	Super-Hatsuga-broccoli	*Brassica oleracea*	Leaf vegetable	Vesicaria	0.120	3.6
53	Radish sprouts	Kaiware	*Raphanus sativus*	Leaf vegetable	Vesicaria	0.848	42.4
54	Water morning glory (Sprout)	Kushin-sai	*Ipomoea aquatic*	Leaf vegetable	Convolvulaceae	0.166	11.3
55	Lettuce	Retasu	*Lactuca sativa*	Leaf vegetable	Asteraceae	0.354	26.6
56	Red leaf lettuce	Sunny-retasu	*Lactuca sativa*	Leaf vegetable	Asteraceae	0.075	5.2
57	Leaf lettuce	Green leaf	*Lactuca sativa*	Leaf vegetable	Asteraceae	0.149	6.5
58	Napa cabbage	Haku-sai	*Brassica rapa*	Leaf vegetable	Vesicaria	0.253	19.0
59	Paprika (red)	Papurika	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.299	11.5
60	Paprika (yellow)	Papurika	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.251	9.9
61	Paprika (orange)	Papurika	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.191	8.1
62	Chicory	Chikori	*Cichorium intybus*	Leaf vegetable	Asteraceae	0.161	9.5
63	Crown daisy	Kiku-na	*Glebionis coronaria*	Leaf vegetable	Asteraceae	0.177	10.6
64	Radish	Radish	*Raphanus sativus*	Root vegetables	Vesicaria	1.223	69.2
65	Scallion	Wakegi	*Allium fistulosum*	Leaf vegetable	Amaryllidaceae	0.120	3.8
66	Parsley	Paseri	*Petroselinum crispum*	Herb	Apiaceae	0.167	4.2
67	Spinach	Horenso	*Spinacia oleracea*	Leaf vegetable	Chenopodiaceae	0.127	4.8
68	Nalta jute	Moroheiya	*Corchorus olitorius*	Leaf vegetable	Tiliaceae Juss.	0.040	0.8
69	Mizuna (potherb mustard)	Mizu-na	*Brassica rapa*	Leaf vegetable	Vesicaria	0.173	7.2
70	Mibuna	Mibu-na	*Brassica rapa*	Leaf vegetable	Vesicaria	0.222	11.7
71	Komatsuna (Japanese mustard spinach)	Komatsu-na	*Brassica rapa*	Leaf vegetable	Vesicaria	0.278	12.3
72	Red cabbage	Aka-kyabetu	*Brassica oleracea*	Leaf vegetable	Vesicaria	0.221	8.1
73	Carrot greens (leaf)	Ninjin-sai	*Daucus carota*	Leaf vegetable	Apiaceae	0.380	18.7
74	Japanese honeywort	Mitsuba	*Cryptotaenia canadensis*	Leaf vegetable	Apiaceae	0.363	16.5
75	Green perilla (leaf)	Oba	*Perilla frutescens*	Leaf vegetable	Lamiaceae	0.138	3.1
76	Japanese parsley	Seri	*Oenanthe javanica*	Leaf vegetable	Apiaceae	0.245	13.1
77	Boston lettuce	Sarada-na	*Lactuca sativab*	Leaf vegetable	Asteraceae	0.134	5.0
78	Japanese horseradish	Wasabi	*Wasabia japonica*	Root vegetables	Vesicaria	9.690	94.4
79	White mushroom	White mushroom	*Agaricus bisporus*	Mushroom	Pleurotaceae	0.772	29.0
80	Green pepper	Shishitou	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.256	9.4
81	Malabar spinach	Tsurumurasaki	*Basella alba*	Leaf vegetable	Basellaceae	0.266	10.6
82	Malabar spinach (leaf)	Tsurumurasaki	*Basella alba*	Leaf vegetable	Basellaceae	0.224	9.5
83	Malabar spinach (stem)	Tsurumurasaki	*Basella alba*	Leaf vegetable	Basellaceae	0.224	14.0
84	Shirona Chinese cabbage	Shiro-na	*Brassica rapa*	Leaf vegetable	Vesicaria	0.311	17.9
85	Garden pea sprouts (Sprout)	Mame-nae	*Pisum sativm*	Leaf vegetable	Fabaceae	0.539	21.9
86	Water morning glory	En-sai (Enkushin)	*Ipomoea aquatic*	Leaf vegetable	Convolvulaceae	0.265	9.8
87	Red giant elephant ear (Petiole)	Beni-zuiki	*Colocasia gigantean*	Stem vegetables	Araceae	0.417	19.5
88	Ginkgo	Ginnan	*Ginkgo biloba*	Nut	Ginkgoaceae	22.841	167.9
89	Chrysanthemum (Petal)	Shokuyo-kiku	*Chrysanthemum morifolium*	Fruit vegetable	Asteraceae	0.096	1.9
90	Jelly ear fungus	Kikurage	*Auricularia auricular*	Mushroom	Auricularaceae	> 10,000	> 10,000
91	Chinese yam	Yamato-imo	*Dioscorea batatas*	Root vegetables	Dioscoreaceae	1.333	13.4
92	Horseradish	Seiyou-wasabi	*Armoracia rusticana*	Root vegetables	Vesicaria	1.234	14.7
93	New ginger	Shin-shouga	*Zingiber officinale*	Root vegetables	Zingiberaceae	0.054	3.8
94	Saltwort	Wakame-okahijiki	*Salsola komarovii*	Leaf vegetable	Chenopodiaceae	0.323	11.5
95	Common ice plant	Barafu	*Mesembryanthemum crystallinum*	Leaf vegetable	Aizoaceae	0.406	33.8
96	Wasabi leaves	Wasabi-na	*Brassica juncea*	Leaf vegetable	Vesicaria	0.199	6.8
97	Perilla (Shiso)	Hojiso	*Perilla frutescens*	Fruit vegetable	Lamiaceae	0.097	1.4
98	Giant elephant ear	Hasu-imo	*Colocasia gigantean*	Stem vegetables	Araceae	0.513	11.5
99	Red rhubarb	Aka-rubabu	*Rheum rhabarbatum*	Stem vegetables	Polygonaceae	0.364	16.3
100	Red rhubarb (no peel)	Aka-rubabu	*Rheum rhabarbatum*	Stem vegetables	Polygonaceae	0.568	29.4

ID	Common Name	Japanese name	Scientific Name	Vesicaria	Family	IC_{50} (mg/mL)	Daily AG Eq (g)
101	Red rhubarb (with peel)	Red-rubaubu	*Rheum rhabarbatum*	Stem vegetables	Polygonaceae	0.107	4.4
102	Shallot	Esharotto	*Allium oschaninii*	Root vegetables	Alliaceae	242.004	2927
103	Cauliflower (flower bud)	Karihurawa	*Brassica oleracea*	Fruit vegetable	Vesicaria	1.039	39.5
104	Cauliflower (stem)	Karifurawa	*Brassica oleracea*	Fruit vegetable	Vesicaria	1.814	71.6
105	Cauliflower (leaf)	Karifurawa	*Brassica oleracea*	Fruit vegetable	Vesicaria	0.251	11.4
106	Turnip (root)	Ko-kabura	*Brassica rapa*	Root vegetables	Vesicaria	0.164	9.1
107	Turnip	Ko-kabura	*Brassica rapa*	Root vegetables	Vesicaria	0.688	44.9
108	Green cucumber	Ao-uri	*Cucumis sativus*	Fruit vegetable	Cucurbitaceae	0.151	12.2
109	Kagahuto cucumber	Kagahuto-kyuri	*Cucumis sativus*	Fruit vegetable	Cucurbitaceae	0.292	24.3
110	Rice eggplant (no peel)	Bei-nasu	*Solanum melongena*	Fruit vegetable	Solanaceae	0.128	5.4
111	Rice eggplant (with peel)	Bei-nasu	*Solanum melongena*	Fruit vegetable	Solanaceae	0.081	3.5
112	Kintoki carrot* (root)	Kintoki-ninjin	*Daucus carota*	Root vegetables	Apiaceae	0.513	41.6
113	Kintoki carrot* (leaf)	Kintoki-ninjin	*Daucus carota*	Root vegetables	Apiaceae	0.207	5.4
114	Red bell pepper	Aka-piman	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.442	12.9
115	Leaf ginger (root)	Ha-shouga	*Zingiber officinale*	Root vegetables	Zingiberaceae	19.328	1159.7
116	Leaf ginger (leaf)	Ha-shouga	*Zingiber officinale*	Root vegetables	Zingiberaceae	46.289	1735.8
117	Mugwort (powder)	Yomogi	*Artemisia indica*	Leaf vegetable	Asteraceae	0.070	0.2
118	Azuki bean	Azuki	*Vigna angularis*	Bean	Fabaceae	0.948	3.1
119	Red kidney beans	Kintoki-mame	*Phaseolus vulgaris*	Bean	Fabaceae	0.771	2.6
120	Black-eyed pea	Sasage	*Vigna unguiculata*	Bean	Fabaceae	0.385	1.2
121	Black soybean	Kuro-mame	*Glycine max*	Bean	Fabaceae	0.333	1.1
122	Soybean	Daizu	*Glycine max*	Bean	Fabaceae	1.835	6.0
123	Black sesame	Kuro-goma	*Sesamun indicum.*	Nut	Pedaliaceae	3.480	11.1
124	White sesame	Shiro-goma	*Sesamun indicum*	Nut	Pedaliaceae	>2800	8845
125	White opium poppy seeds (seed)	Shirokeshi-no-mi	*Papaver somniferum*	Nut	Papaveraceae	>10,000	>10,000
126	Almond	Almond	*Amygdalus communis*	Nut	Rosaceae	324.460	1000
127	Cashew nuts	Kashu-nattsu	*Anacardium occidele*	Nut	Anacardiaceae	>10,000	>10,000
128	Walnut	Kurumi	*Juglans regia*	Nut	Juglandaceae	0.169	0.5
129	Pumpkin seeds (seed)	Kawanashi-kabocha-no-tane	*Cucurbita sp.*	Nut	Cucurbitaceae	>10,000	>10,000
130	Peanut	Rakkasei	*Arachis hypogaea.*	Nut	Fabaceae	3.891	17.3
131	Polished rice A	Haku-mai A	*Oryza sativa*	Grain	Poaceae (Gramineae)	>10,000	>10,000
132	Red-kernelled rice	Aka-mai	*Oryza sativa*	Grain	Poaceae (Gramineae)	0.433	1.3
133	Reddish black rice	Kuro-mai	*Oryza sativa*	Grain	Poaceae (Gramineae)	0.475	1.4
134	Rolled barley	Oshi-mugi	*Hordeum vulgare*	Grain	Poaceae (Gramineae)	119.751	359.3
135	Pearl barley	Hato-mugi	*Coix lacryma-jobi*	Grain	Poaceae (Gramineae)	>2600	7935
136	Pistachio	Pisutachio	*Pistacia vera*	Nut	Anacardiaceae	>10,000	>10,000
137	Foxtail millet	Awa	*Setaria italica*	Grain	Poaceae (Gramineae)	373.134	1119
138	Japanese millet	Hie	*Echinochloa esculenta*	Grain	Poaceae (Gramineae)	>10,000	>10,000
139	Proso millet	Kibi	*Panicum miliaceum*	Grain	Poaceae (Gramineae)	>10,000	>10,000
140	Amaranth	Amaransasu	*Amaranthus sp.*	Grain	Amaranthaceae	677.645	2033
141	Quinoa	Kinusu	*Chenopodium quinoa*	Grain	Chenopodiaceae	11.108	33.3
142	Pecan nuts	Pecan	*Carya illinoinensis*	Nut	Juglandaceae	0.141	0.4
143	Lady's thumb	Tade	*Polygonum hydropiper*	Leaf vegetable	Polygonaceae	0.072	1.7
144	Polished rice B	Haku-mai B	*Oryza sativa*	Grain	Poaceae (Gramineae)	>10,000	>10,000

ID	Common Name	Japanese name	Scientific Name	Vesicaria	Family	IC_{50} (mg/mL)	Daily AG Eq (g)
145	Unpolished rice B	Gen-mai	*Oryza sativa*	Grain	Poaceae (Gramineae)	203.557	610.7
146	Rice B bran	Nuka	*Oryza sativa*	Grain	Poaceae (Gramineae)	1.409	4.2
147	Garbanzo	Garbanzo	*Cicer arietinum*	Bean	Fabaceae	405.765	1325
148	Lentil	Renzu-mame	*Lens culinaris*	Bean	Fabaceae	8.583	27.7
149	Bay leaves	Bay leaf	*Laurus nobilis*	Herb	Lauraceae	0.090	0.3
150	Water chestnut (seed)	Hishi	*Trapa japonica*	Nut	Trapaceae	1.304	16.4
151	Water chestnut (seed coat)	Hishi	*Trapa japonica*	Nut	Trapaceae	0.017	0.3
152	Butterscotch mushroom	Nameko	*Pholiota microspore*	Mushroom	Strophariaceae	1.075	38.4
153	Chestnut (seed)	Kuri	*Castanea crenata*	Nut	Fagaceae	2.378	16.6
154	Chestnut (outer skin)	Kuri	*Castanea crenata*	Nut	Fagaceae	0.015	0.1
155	Chestnut (soft layer)	Kuri	*Castanea crenata*	Nut	Fagaceae	0.030	0.2
156	Chrysanthemum (receptacle)	Shokuyo-kiku	*Chrysanthemum morifolium*	Fruit vegetable	Asteraceae	0.142	3.6
157	Pea (pod)	Endou-mame	*Pisum sativum*	Bean	Fabaceae	0.638	18.8
158	Pea (bean)	Endou-mame	*Pisum sativum*	Bean	Fabaceae	>10,000	>10,000
159	Turnip rape	Nanohana	*Brassica rapa*	Fruit vegetable	Vesicaria	0.539	19.3
160	Horikawa gobo*	Horikawa-gobou	*Arctium lappa*	Root vegetables	Asteraceae	0.187	3.0
161	Shogoin turnip*	Seigoin-kabura	*Brassica rapa*	Root vegetables	Vesicaria	3.883	200.8
162	Ashitaba	Ashitaba	*Angelica keiskei*	Leaf vegetable	Apiaceae	0.143	4.1
163	Kyoto eggplant*	Nasubi "Kyo-shizuku"	*Solanum melongena*	Fruit vegetable	Solanaceae	0.608	27.2
164	Ostrich fern	Kogomi	*Matteuccia struthiopteris*	Leaf vegetable	Athyriaceae	0.181	5.8
165	Kamo eggplant*	Kamo-nasu	*Solanum melongena*	Fruit vegetable	Solanaceae	0.420	22.9
166	Manganzi chile pepper* (red)	Mannganji-tougarashi	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.345	9.5
167	Manganzi chile pepper* (green)	Manganji-tougarashi	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.476	16.6
168	Manganzi chile pepper* (mixed)	Manganji-tougarashi Mix	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.695	22.9
169	Takagamine chile pepper*	Takagamine-tougarashi	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.440	12.5
170	Husimiamanaga Chile pepper* (red)	Fushimi-amanaga-tougarashi	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.412	7.9
171	Husimiamanaga Chile pepper* (rreen)	Fushimi-amanaga-tougarashi	*Capsicum annuum*	Fruit vegetable	Solanaceae	0.405	10.3
172	Super Yuga tomato*	Super Yuga tomato	*Solanum lycopersicum*	Fruit vegetable	Solanaceae	0.284	12.3
173	Renaissance tomato*	Runessansu tomato	*Solanum lycopersicum*	Fruit vegetable	Solanaceae	0.493	22.4
174	Kyo-akane tomato*	Kyo-akane tomato	*Solanum lycopersicum*	Fruit vegetable	Solanaceae	0.366	12.6
175	Kyo-temari tomato*	Kyo-temari tomato	*Solanum lycopersicum*	Fruit vegetable	Solanaceae	0.521	23.0
176	Rucola	Rukkora	*Eruca vesicaria*	Herb	Vesicaria	0.331	16.0
177	Sweet basil	Sweet bajiru	*Ocimum basilicum*	Herb	Lamiaceae	0.189	5.6
178	Common sage	Seiji	*Salvia officinalis*	Herb	Lamiaceae	0.508	10.9
179	Dill	Diru	*Anethum graveolens*	Herb	Apiaceae	0.219	4.8
180	Italian parsley	Italian-pasure	*Petroselinum neapolitanum*	Herb	Apiaceae	0.390	5.5
181	Chervil	Chabiru	*Anthriscus cerefolium*	Herb	Apiaceae	0.263	4.7
182	Thyme	Taimu	*Thymus sp.*	Herb	Lamiaceae	0.186	3.7
183	Sweet leaf	Sutebia	*Stevia rebaudiana*	Herb	Asteraceae	0.186	3.6
184	Lemon balm	Lemon baumu	*Melissa officinalis*	Herb	Lamiaceae	0.124	2.6
185	Peppermint	Peppermint	*Mentha x piperita*	Herb	Lamiaceae	0.360	9.2
186	Spearmint	Spearmint	*Mentha spicata*	Herb	Lamiaceae	0.144	3.1
187	Rosemary	Rosemary	*Rosmarinus officinalis*	Herb	Lamiaceae	0.059	0.8

IC_{50}: 50% inhibitory concentration. Mean of three measurements for each sample shown. *Kyoto regional vegetable

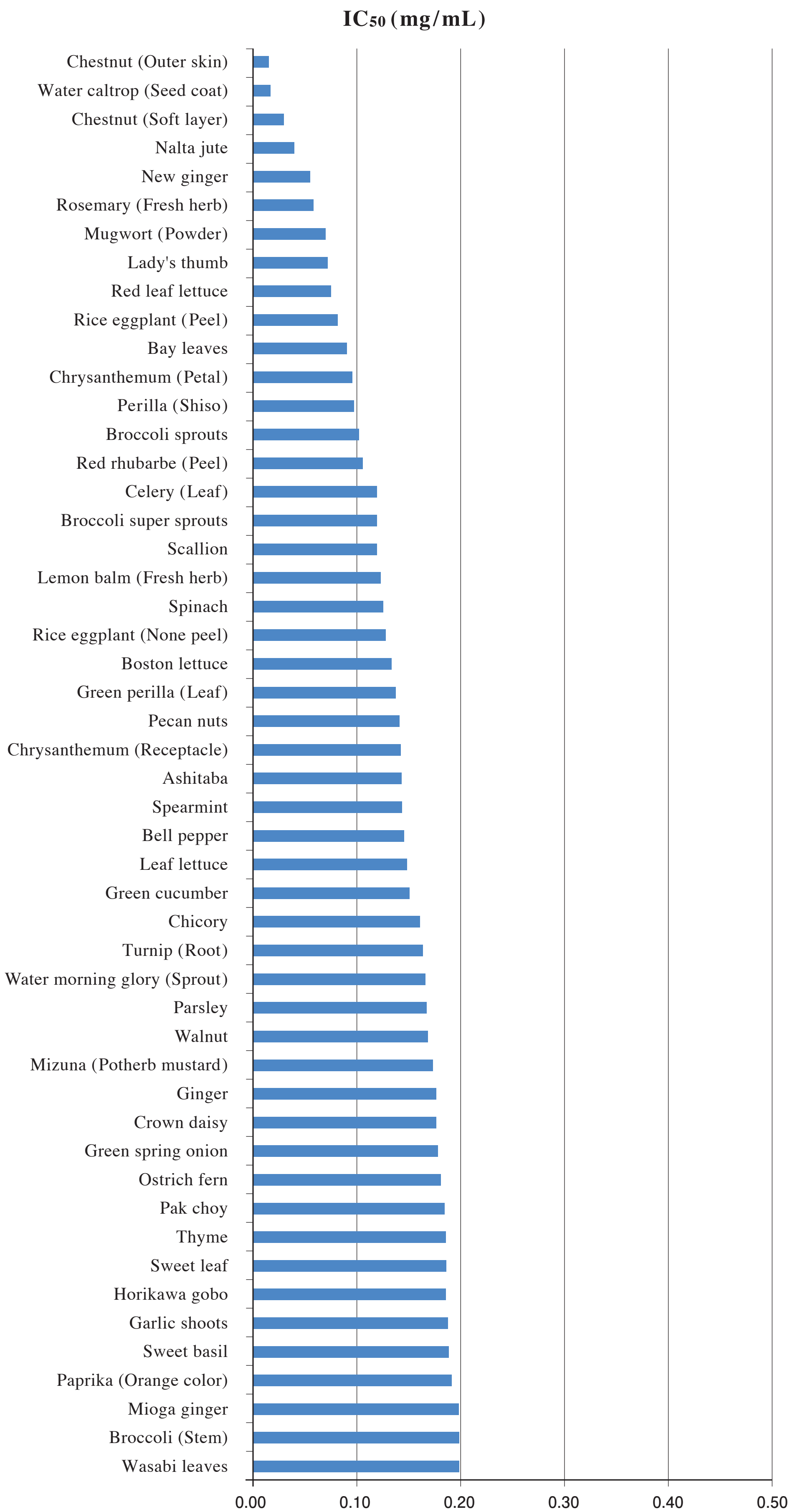
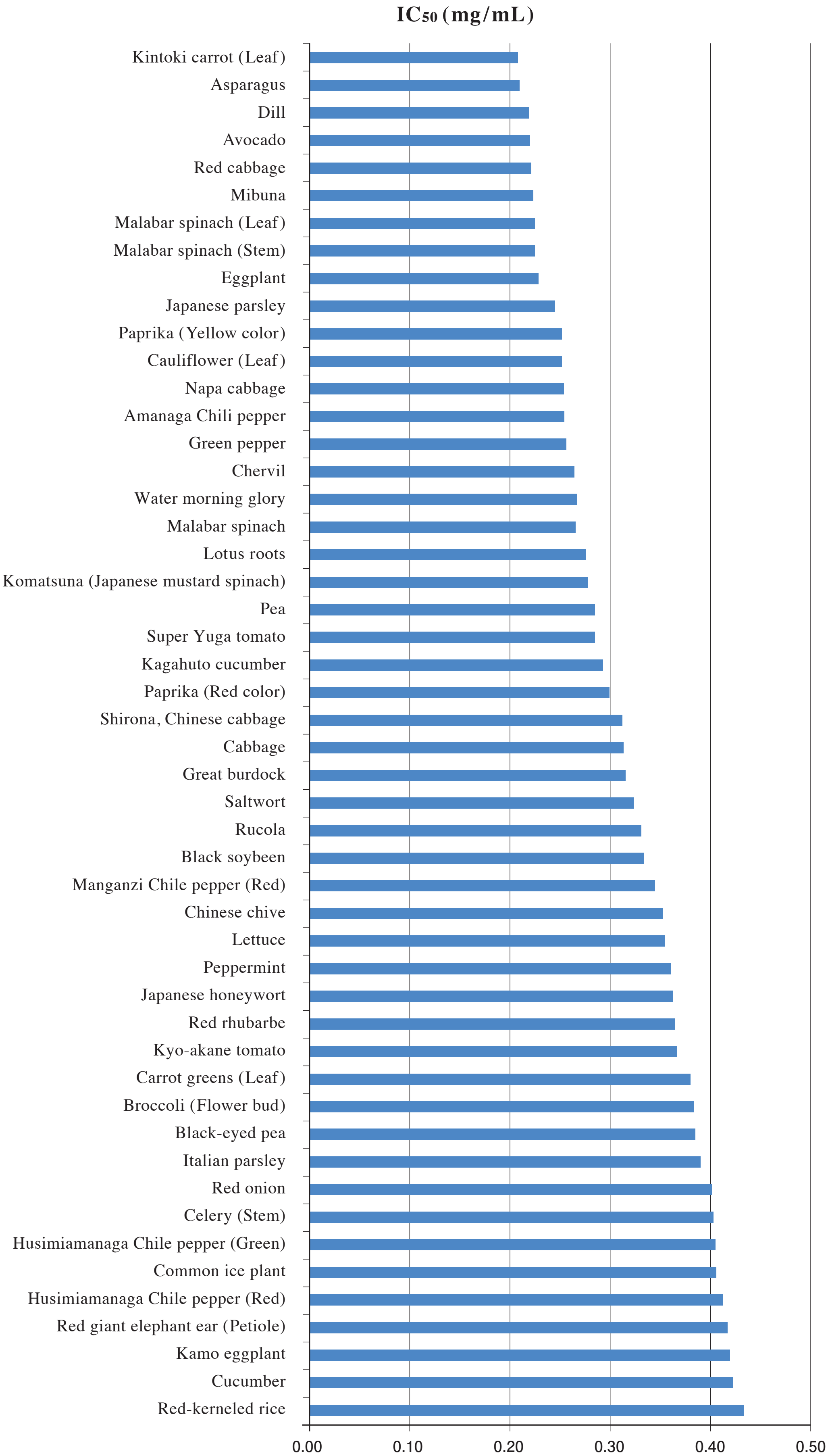


Fig 1. **Antiglycation activity ranking of top 100 of 187 vegetable varieties used IC_{50}: 50% inhibitory concentration.**


IC50 (mg/mL)
Kintoki carrot (Leaf)
Asparagus
Dill
Avocado
Red cabbage
Mibuna
Malabar spinach (Leaf)
Malabar spinach (Stem)
Eggplant
Japanese parsley
Paprika (Yellow color)
Cauliflower (Leaf)
Napa cabbage
Amanaga Chili pepper
Green pepper
Chervil
Water morning glory
Malabar spinach
Lotus roots
Komatsuna (Japanese mustard spinach)
Pea
Super Yuga tomato
Kagahuto cucumber
Paprika (Red color)
Shirona, Chinese cabbage
Cabbage
Great burdock
Saltwort
Rucola
Black soybeen
Manganzi Chile pepper (Red)
Chinese chive
Lettuce
Peppermint
Japanese honeywort
Red rhubarbe
Kyo-akane tomato
Carrot greens (Leaf)
Broccoli (Flower bud)
Black-eyed pea
Italian parsley
Red onion
Celery (Stem)
Husimiamanaga Chile pepper (Green)
Common ice plant
Husimiamanaga Chile pepper (Red)
Red giant elephant ear (Petiole)
Kamo eggplant
Cucumber
Red-kerneled rice
0.00
0.10
0.20
0.30
0.40
0.50

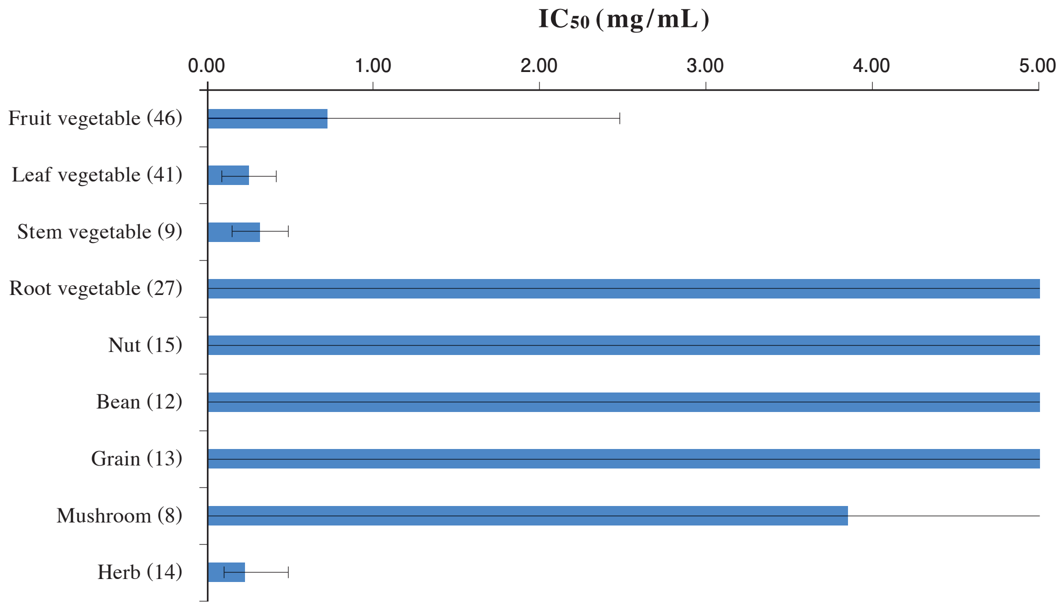


Fig 2. **Antiglycation activity by vegetable category**

Parentheses indicate number of samples included in vegetable category. IC_{50}: 50% inhibitory concentration. Bars indicate standard deviation.

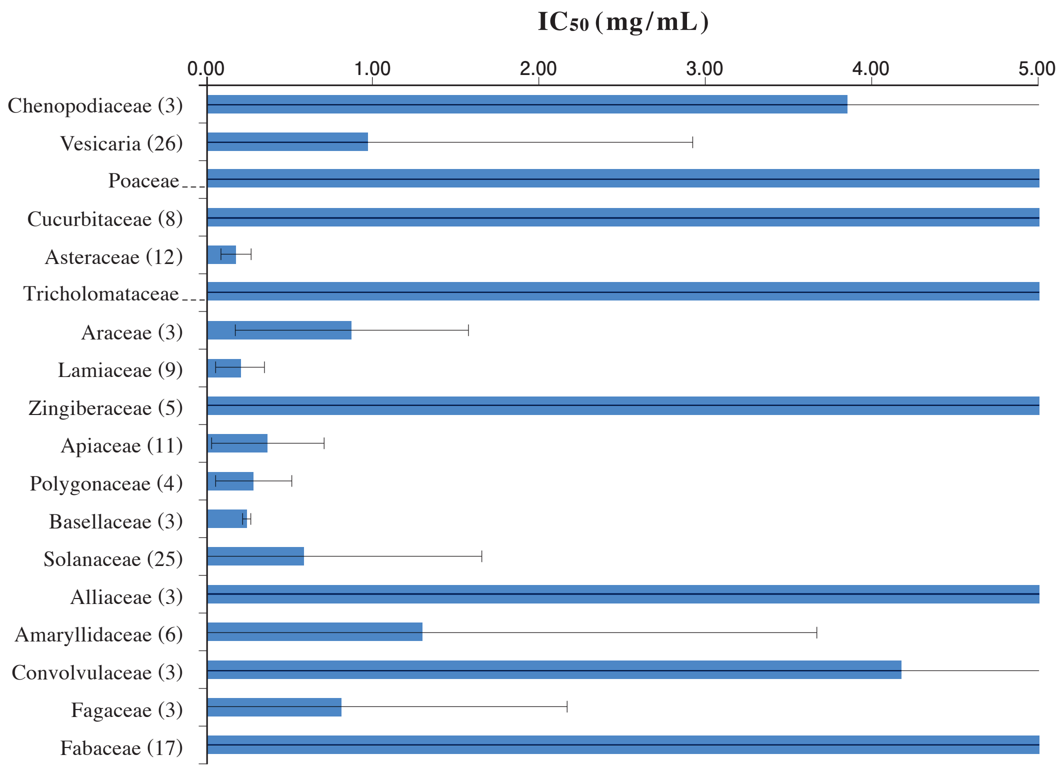


Fig 3. **Antiglycation activity by taxonomic vegetable family**

Parentheses indicate number of samples included in vegetable family. IC_{50}: 50% inhibitory concentration. Bars indicate standard deviation.

Discussion

Many reports state that tea leaves [4,8], herbs and spices [9,10,11], herbal remedies [12], and other natural, plant-based materials have an antiglycation effect *in vitro* or in animal experiments. These effects are attributed primarily to polyphenols contained in plants. Our experiment using dried powders produced from 187 different commercial vegetable samples to measure the inhibitory effect of these substances on AGE formation in an *in vitro*, glucose HSA reaction system. The results showed that vegetables in the Asteraceae, Lamiaceae, Basellaceae, and Polygonaceae families include varieties with potent inhibitory action on AGE formation.

Plants in the Asteraceae family are one thriving taxonomic group among the angiosperms and include more than 20,000 species worldwide. Asterid classifications include the Asteraceae, Lamiaceae, Apiaceae, and Solanaceae families, and the mean IC_{50} of these four families is potent, at less than 0.6 mg/mL, suggesting that plants included in the asterid clade contain a characteristic component having strong antiglycative action. Edible chrysanthemums (*Chrysanthemum indicum or C. morifolium*) are a material with an extensive dietary history of traditional use for detoxification and relief of fever, anti-inflammatory properties, and ophthalmic problems in general (*e.g.*, blurry vision, dryness, itching, diminished eyesight, eyestrain, and bloodshot eyes). Reports from recent research state that tetracoumaroyl spermine found in edible chrysanthemums is effective in diabetic renal dysfunction and diabetic neuropathy, acts to increase production of intracellular glutathione [13], and strongly inhibits [14] AGE formation as indexed by carboxymethyl-lysine (CML) and pentosidine. Results verified through our *in vitro* glycation reaction system for evaluating the antiglycation effect of dried petal powder from edible chrysanthemums (*C. morifolium*) showed that purple chrysanthemum powder significantly inhibited formation of fluorescent AGEs, specifically, 3-deoxyglucosone (3DG), pentosidine, and CML [7]. We attribute this phenomenon to the substantial content of polyphenols (luteolin, anthocyanidin), an active ingredient, in chrysanthemums, and likewise, to chlorogenic acid.

Rosids classifications include Fabaceae and Cucurbitaceae, and the mean IC_{50} value of these two families was weak, at more than 10,000 mg/mL, suggesting that rosids have weak antiglycation activity. Monocot classifications include Alliaceae, Poaceae (Gramineae), and Zingiberaceae, and the mean IC_{50} value of these families also showed weak activity, at more than 10,000 mg/mL, indicating a pattern of weak antiglycation activity in monocots as well.

Intake of 150 mg-300 mg/day of AG, a glycation inhibitor, has been shown to have a progression-inhibiting effect on diabetic nephropathy [15], and we calculated the daily intake of various vegetable samples needed to obtain antiglycation activity equivalent to that of AG (***Fig. 4***). Given that the 2000 National Nutrition Survey found a daily mean vegetable intake of 293.8 g/day among males and 275.3 g/day among females, we found that 157 of the 187 vegetable samples in our study could provide daily antiglycation activity equivalent to that of AG. In other words, we found that approximately 84% of the vegetable samples we studied for this work provide effective antiglycation in an ordinary diet. Thus, a commitment to consuming a target amount of 350 g/day of various vegetables, regarded as a preferable figure for required daily vegetable intake, may provide antiglycation activity equivalent to that of AG and may also go beyond inhibition of aging to play a role in preventing the onset and progression of lifestyle-related diseases.

Conclusion

We used dried powders produced from vegetables to measure inhibitory effect on AGE formation in an *in vitro*, glucose HSA reaction system. The results from comparison showed that vegetables in the Asteraceae, Lamiaceae, Basellaceae, and Polygonaceae families include species with a potent inhibitory effect on AGE formation, suggesting that these species may contain components with antiglycation activity. We believe that this information is useful for creating dietary regimens to counter glycation stress.

Acknowledgements

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Conflict of Interest Statement

The authors state that performance of this study entailed no issues representing a conflict of interest.

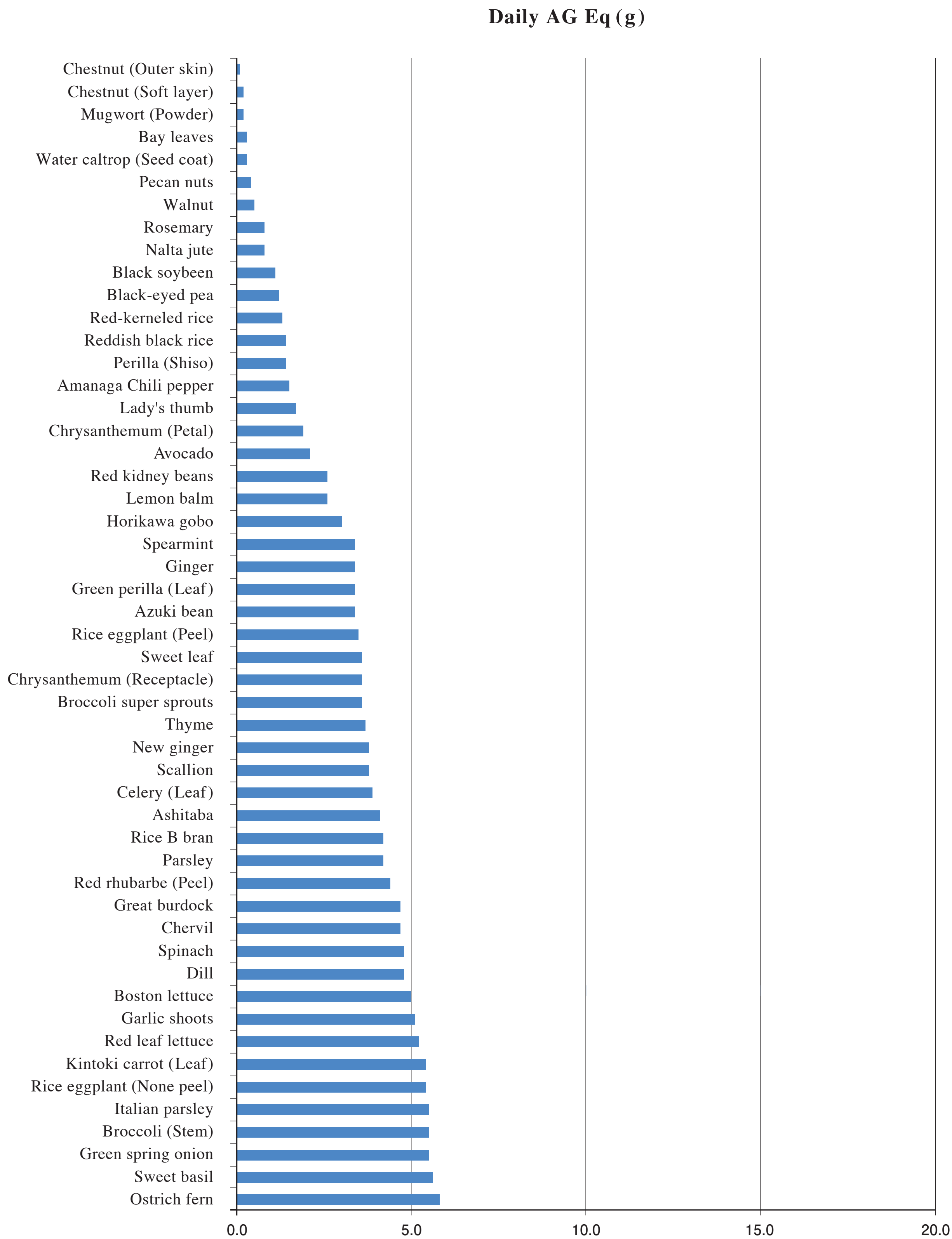
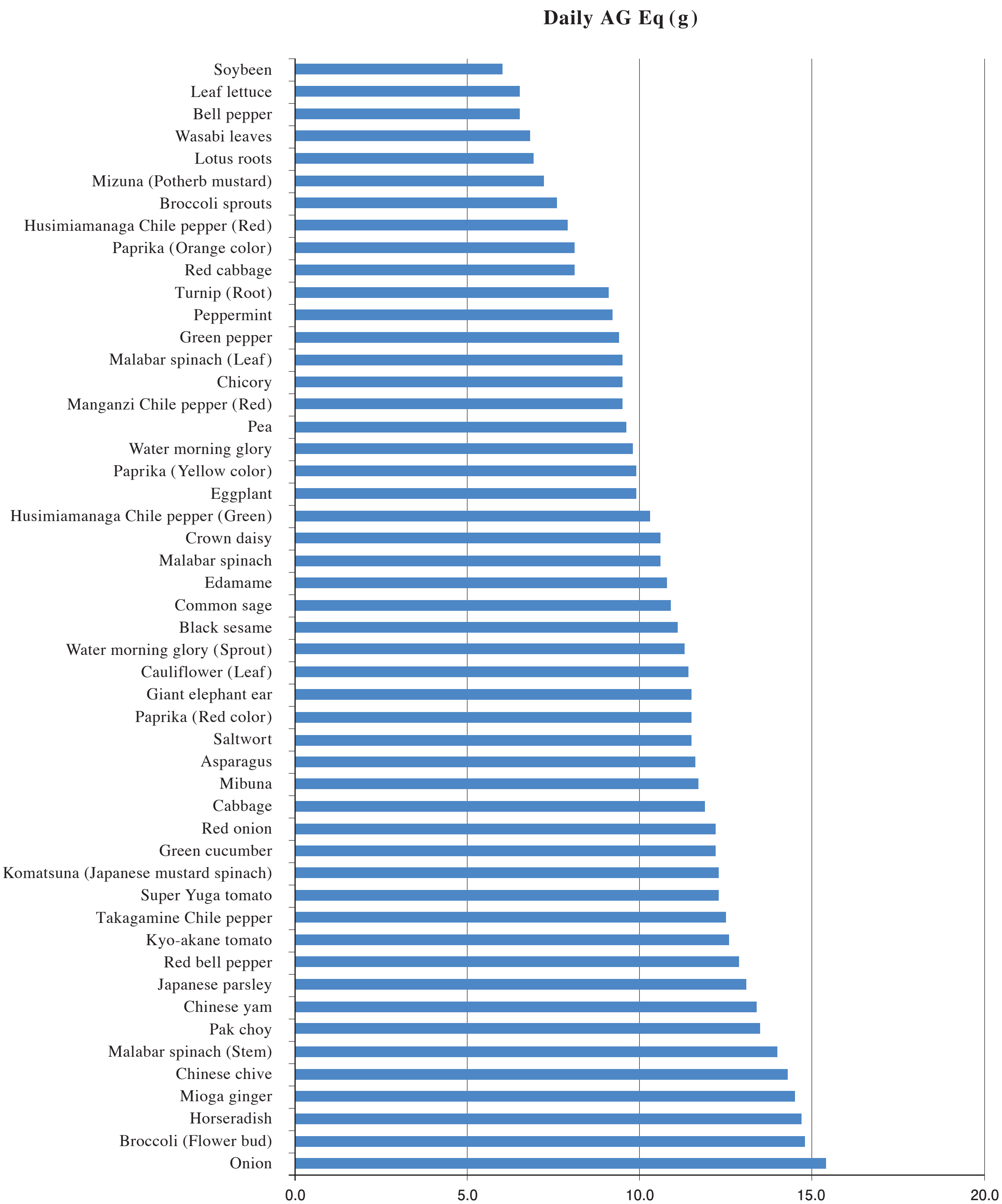


Fig 4. **Ranking of required vegetable intake to manifest AG (300 mg)- equivalent effect (top 100)**

AG, aminoguanidine; Eq, equivalent.


Daily AG Eq (g)
Soybeen
Leaf lettuce
Bell pepper
Wasabi leaves
Lotus roots
Mizuna (Potherb mustard)
Broccoli sprouts
Husimiamanaga Chile pepper (Red)
Paprika (Orange color)
Red cabbage
Turnip (Root)
Peppermint
Green pepper
Malabar spinach (Leaf)
Chicory
Manganzi Chile pepper (Red)
Pea
Water morning glory
Paprika (Yellow color)
Eggplant
Husimiamanaga Chile pepper (Green)
Crown daisy
Malabar spinach
Edamame
Common sage
Black sesame
Water morning glory (Sprout)
Cauliflower (Leaf)
Giant elephant ear
Paprika (Red color)
Saltwort
Asparagus
Mibuna
Cabbage
Red onion
Green cucumber
Komatsuna (Japanese mustard spinach)
Super Yuga tomato
Takagamine Chile pepper
Kyo-akane tomato
Red bell pepper
Japanese parsley
Chinese yam
Pak choy
Malabar spinach (Stem)
Chinese chive
Mioga ginger
Horseradish
Broccoli (Flower bud)
Onion
0.0
5.0
10.0
15.0
20.0

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