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# An *in vitro* screening study of food functions among vegetable cultivars consumed in Japan

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## ABSTRACT

**Aim:** Ingesting vegetables plays a major role in health maintenance and disease prevention. Therefore, this study aimed to identify hitherto unknown food functionalities among various vegetable cultivars consumed in Japan.

**Methods:** Water-soluble vegetable extracts were screened for various types of functionality such as antioxidant action, the inhibition of platelet aggregation, blood coagulation, and the enzymatic actions of α-glucosidase and pancreatic lipase. Antioxidant activity was measured as the ability to scavenge 1,1-diphenyl-2-picrylhydrazine (DPPH) radicals and the inhibitory activity of rat intestinal α-glucosidase was evaluated by measuring amounts of glucose produced from maltose. Lipase activities were measured using porcine pancreatic lipase and synthetic substrate. Platelet aggregation was measured using bovine whole blood stimulated with collagen. Plasma coagulation activities were measured using human plasma. Coagulation was initiated using intrinsic and extrinsic coagulation initiators and coagulation times were measured using a coagulometer.

**Results:** All extracts from cabbage, onion, eggplant, tomato, green pepper, Japanese radish, and Chinese cabbage and their cultivars exerted antioxidant activities. All vegetable extracts had weak α-glucosidase inhibitory activity, but one Chinese cabbage cultivar had significantly more of such activity. All vegetable extracts had very weak ability to inhibit pancreatic lipase and did not inhibit platelet aggregation or affect intrinsic and extrinsic blood coagulation activity.

**Conclusion:** Although further investigations are needed, the present findings provide fundamental information about the functionality of some vegetables consumed in Japan.

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Antioxidant; α-glycosidase; lipase; platelet; coagulation; lifestyle diseases

## Introduction

Vegetable consumption is thought to prevent or cure many ailments; and the relationships between vegetables and health have been studied in detail for decades. The amounts of fruit and vegetable consumption are significantly associated with protection against coronary heart disease [1]; and vegetable intake is associated with protection against various types of cancer [2]. A systematic review and meta-analysis have shown that reduced fruit and vegetable intake plays a critical role in the development of asthma and allergies [3]. Fruits and vegetables contain various amounts of phytochemicals that exert anti-inflammatory effects [4]. A screen of the experimental antithrombotic effects of fruits and vegetables using tests of shear-induced thrombosis *ex vivo* (global thrombosis test, GTT), and laser-induced thrombosis *in vivo* has shown that the antithrombotic activities are independent of polyphenolic content or antioxidant activities [5].

All vegetables contain micronutrients, minerals, vitamins, and many other valuable components, and the components and their proportions differ among cultivars of specific vegetables [6]. Cultivars and the wild relatives of many vegetable crops harbor

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a considerable diversity of phenolic acid contents [6], and breeding conditions affect the contents of bioactive phenolic acids in vegetables [7].

Here, we screened the effects of water-soluble vegetable extracts on platelet aggregation, blood coagulation, and α-glucosidase and pancreatic lipase activities, and antioxidant action. We also assessed whether or not these actions and effects differ among cultivars of vegetables that were considered likely to be effective. The purpose of our study was to identify hitherto unknown food functionality in various vegetable cultivars consumed in Japan. Our screening found some possible food functionalities in the tested vegetables.

## Materials and Methods

### *Vegetables and cultivars*

All vegetables were cultivated at the Institute of Vegetable and Floriculture Science, NARO, Mie, Japan. Vegetable samples were collected between 2012 and 2015 at appropriate times. Table 1 shows the names and cultivars of the vegetables assessed herein that are habitually consumed in Japan, except F1Jenost, which is a Russian cultivar of Chinese cabbage.

**Table 1.** Various vegetables and cultivars assessed in present study.

| Types of vegetable | Cultivar |
|---|---|
| Cabbage (*Brassica oleracea*) | Kinkei No.201<br>Kanroku<br>Satuki-Joō |
| Onion (*Allium cepa*) | Shōunan red<br>Amagashi No.2 |
| Eggplant (*Solanum melongena*) | Senryo No.2<br>Anominori<br>Shōyaōnaga<br>Shisui<br>Tayanasu<br>Hetamurasaki |
| Tomato (*Solanum lycopersicum*) | Momotaro<br>Momotaro 8<br>Zuiei<br>Fruit<br>ACE |
| Green pepper (*Capsicum annuum*) | Kyōsuzu<br>Kyōmatsuri<br>Musashi |
| Japanese radish (*Raphanus sativus*) | Taibyōsōbutori<br>Kiributomiyashige<br>Karami 199<br>Fuyudorishōgoin<br>Sakurajima<br>Daikōhō |
| Chinese cabbage (*Brassica rapa*) | Haregi<br>Kanjiro<br>F1Jenost |

### *Preparation of vegetable extracts*

Lyophilized vegetable powder (1 g) from each cultivar was added to 10 ml of hexane/dichloromethane (50:50, v/v), vortex-mixed three times and then separated by centrifugation at 3,500 rpm and the supernatant was decanted. The residue was resuspended in 10 ml of hexane/dichloromethane (50:50, v/v) and the same process was repeated. The two supernatants were mixed, dried *in vacuo*, resuspended in 10 ml of methanol/water/acetic acid (90:9.5:0.5, v/v), then otherwise the same process was repeated as described above. Supernatants from the two separations were combined, made up to a volume of 25 ml and stored at −20°C.

### *Antioxidant activity*

Antioxidant activity was measured as the ability to scavenge 1,1-diphenyl-2-picrylhydrazine (DPPH) radicals as described by Blois [8] with minor modifications. A 10-µl portion of each extract was added to 90 µl of DPPH (0.08 mg/ml). The mixture was then vigorously shaken and incubated at room temperature for 30 minutes in darkness. Absorbance at 540 nm was measured using an iMark UV microplate reader (Bio-Rad, Hercules, CA, USA). The control contained all reagents without the sample, and methanol:water:acetic acid (90:9.5:0.5) served as the blank. We calculated the antioxidant activity as:

$$(Ac - As)/Ac \times 100\ (\%),$$

where Ac is the absorbance of DPPH solution without extract and As is the absorbance of the sample in reaction mixture. Trolox served as the reference control.

### *Ability to inhibit α-glucosidase*

Rat intestinal acetone powder (1 g; Sigma-Aldrich, St. Louis, MO, USA) was suspended in 10 ml of 0.1 M sodium phosphate buffer (pH 7.0) and then stirred for 30 minutes on ice. The suspension was separated by centrifugation (12,000 rpm × 10 minutes, 4°C) and the supernatant containing crude enzyme was stored at −80°C. Inhibitory activity was assessed as follows: Each extract (50 µl) was mixed with 100 µl of maltose (250 mM stock solution) and added to 240 µl of distilled water, then stirred and heated at 37°C for 5 minutes. Enzyme solution (25 µl) was added to the mixture and the enzymatic reaction proceeded for 40 minutes at 37°C. The reaction was stopped by adding 500 µl of 0.2 M sodium carbonate, and the amount of produced glucose was

measured using Glucose C-test Wako kit (Wako Pure Chemical Industries Ltd., Tokyo, Japan).

The amount of α-glucosidase inhibitory activity was calculated as:

$$(Ac - As)/Ac \times 100\ (\%),$$

where Ac is the absorbance of the control and As is the absorbance of the sample in reaction mixture.

### *Lipase inhibitory activity*

Lipase activity was measured using Lipase kit S (DS Pharma Biomedical, Osaka, Japan). Porcine pancreatic lipase (5 µl of 0.5 mg/ml) (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) was incubated with 5 µl of each vegetable extract for 15 minutes at room temperature (24 ± 2°C). Developer (95 µl) and elastase inhibitor solutions (2 µl) were added and the mixture was incubated for 5 minutes at 30°C. Thereafter, substrate solution (10 µl) was added and the mixture was incubated for 30 minutes at 30°C. The reaction was stopped and then absorbance was measured at 405 nm using a UV microplate reader as described above.

Lipase inhibitory activity was calculated as:

$$(Ac - As)/Ac \times 100\ (\%),$$

where Ac is the absorbance of the control and As is the absorbance of the sample in reaction mixture.

### *Platelet aggregation assays*

Platelets in whole bovine blood were evaluated using a KX-21 blood cell counter (Sysmex, Kobe, Japan) as described [9, 10]. In brief, bovine (1 ml) whole blood samples were incubated with test samples (10 µl) in polypropylene tubes at room temperature (24 ± 2°C) for 30 minutes and then platelet aggregation was induced by adding 20 µl of collagen solution (4 µg/ml). The tubes were then gently and continuously mixed at room temperature for 20 seconds and platelets were counted three times at intervals of 70 seconds. Platelet counts are expressed as ratios (%) of the initial counts in the presence of vehicle only.

Platelet aggregation rates (%) were calculated as:

$$[100 - \text{remaining platelets (\%) (sample)}/ 100 - \text{remaining platelets (\%) (Control)}] \times 100\ (\%).$$

A reduced aggregation rate indicated that an extract included active substances.

### *Coagulation assays*

Prothrombin time (PT) and activated partial prothrombin time (APTT) were measured using Thromborel S (Dade Behring, Marburg, Germany) and Thrombocheck APTT (Sysmex, Kobe, Japan), respectively, according to the manufacturer's instructions. Vegetable extracts (5 µl) were incubated with 100 µl of human standard plasma (Sysmex, Kobe, Japan); and the coagulation reaction was started by adding 100 µl of Thromborel S and 25 µl of 25 mM $CaCl_2$ for PT, or Thrombocheck APTT and 25 µl of 25 mM $CaCl_2$ for APTT.

The amount of time required to form clots was determined using a KC4A coagulometer (Amelung, Lemgo, Germany). The plasma coagulation time was adjusted to about 350 and 250 seconds for the respective PT and APTT controls, respectively. A prolonged coagulation time indicated that the extract included active substances.

### *Statistical analysis*

All experiments proceeded in triplicate ($n$ = 3). All data are reported as means ± standard deviation (SD). Statistical differences between extracts and controls were determined using a one-way analysis of variance (ANOVA) followed by Tukey tests.

## Results

### *Antioxidant activity*

Antioxidants protect cells against damage by free radicals that contribute to the development of cancer and various diseases. The present study assessed the antioxidant activities of various vegetables and their cultivars as the ability to scavenge DPPH radicals (Table 2). All tested extracts of vegetables and their cultivars had similarly effective antioxidant activities, but these were particularly abundant in Japanese radish (Taibyōsōbutori) and onion (Amagashi No. 2).

### *Effects of vegetable extracts on α-glycoside activity*

We initially assessed the α-glucosidase inhibitory activity of one representative cultivar of each vegetable and found weak α-glycosidase inhibitory activity in all of them (Table 3), but tomatoes and Japanese radishes had significantly more and Chinese cabbage (Haregi) had the most. We then found that three Chinese cabbage cultivars had higher levels of α-glycosidase inhibitory activities with Haregi being the highest, followed by F1Jenost and Kanjiro in that order.

**Table 2.** Antioxidant activities of vegetables.

| Vegetable | Cultivars | Antioxidant activity (%)* |
|---|---|---|
| Cabbage | Kinkei No.201 | 64.5 ± 11.1 |
| | Kanroku | 60.1 ± 7.5 |
| | Satuki-Joō | 61.7 ± 7.5 |
| Onion | Shōnan red | 67.3 ± 4.3 |
| | Amagashi No.2 | 76.5 ± 5.0 |
| Eggplant | Senryo No.2 | 59.3 ± 4.9 |
| | Anominori | 65.9 ± 7.6 |
| | Shōyaōnaga | 68.3 ± 9.4 |
| | Shisui | 61.4 ± 5.4 |
| | Tayanasu | 58.9 ± 6.5 |
| | Hetamurasaki | 64.5 ± 10.4 |
| Tomato | Momotaro | 58.8 ± 5.7 |
| | Momotaro 8 | 56.8 ± 5.9 |
| | Zuiei | 58.5 ± 8.1 |
| | Fruit | 54.4 ± 7.9 |
| | ACE | 56.9 ± 6.9 |
| Green pepper | Kyōsuzu | 61.9 ± 8.6 |
| | Kyōmatsuri | 60.2 ± 4.7 |
| | Musashi | 62.3 ± 7.9 |
| Japanese radish | Taibyōsōbutori | 84.2 ± 7.3 |
| | Kiributomiyashige | 69.2 ± 6.8 |
| | Karami 199 | 60.9 ± 4.8 |
| | Fuyudorishōgoin | 54.3 ± 6.9 |
| | Sakurajima | 57.4 ± 6.2 |
| | Daikōhō | 56.3 ± 6.5 |
| Chinese cabbage | Haregi | 80.5 ± 4.4 |
| | Kanjiro | 74.8 ± 4.4 |
| | F1Jenost | 62.6 ± 6.3 |
| Trolox | 5 nM | 72.8 ± 19.2 |
| | 10 nM | 98.6 ± 9.8 |

Values are expressed as means ± SD ($n$ =3).
*All values significantly different versus without extract ($p < 0.05$).

**Table 3.** Ability of vegetables to inhibit α-glucosidase.

| Vegetable | Cultivars | Inhibition (%) |
|---|---|---|
| Cabbage | Kinkei No.201 | 21.3 ± 6.1 |
| Onion | Shōnan red | 33.7 ± 1.7 |
| Eggplant | Senryo No.2 | 21.6 ± 24.6 |
| Tomato | Momotaro | 40.5 ± 9.9* |
| Green pepper | Kyōsuzu | 36.0 ± 4.6 |
| Japanese radish | Taibyōsōbutori | 37.8 ± 8.1* |
| Chinese cabbage | Haregi | 53.1 ± 10.0* |
| | Kanjiro | 33.5 ± 5.0 |
| | F1Jenost | 40.6 ± 5.4* |

Values are expressed as means ± SD ($n$ = 3).
*Significantly different versus without extract $p < 0.05$.

**Table 4.** Ability of vegetables to inhibit lipase.

| Vegetable | Cultivar | Inhibition (%) |
|---|---|---|
| Cabbage | Kinkei No.201 | 10.9 ± 5.4 |
| Onion | Shōnan red | 12.7 ± 1.3 |
| Eggplant | Senryo No.2 | 13.6 ± 9.5 |
| Tomato | Momotaro | 24.9 ± 11.5 |
| Green pepper | Kyōsuzu | 11.8 ± 6.7 |
| Japanese radish | Taibyōsōbutori | 20.0 ± 14.5 |
| Chinese cabbage | Haregi | 19.3 ± 7.5 |

Control and extracts do not significantly differ ($p > 0.05$).

### *Effects of vegetable extract on lipase activity*

The present results showed that the inhibition rates of almost all vegetable extracts were < 20%. Components that significantly inhibit lipase activity might not be water-soluble (Table 4).

### *Effect of vegetables extracts on platelet aggregation, PT and APTT*

The effects of candidate platelet aggregation inhibitors are generally assessed by measuring platelet aggregation activity *in vitro*. However, none of the vegetable extracts tested herein had platelet aggregation inhibitory activities under our conditions (Table 5) or prolonged PT and APTT. That is, none of the vegetable extracts tested under our conditions contained compounds that inhibited or interacted with factors involved in the extrinsic, intrinsic, and common blood coagulation pathways (Table 5).

## Discussion

We screened the effects of water-soluble vegetable extracts on platelet aggregation, blood coagulation, α-glucosidase and pancreatic lipase activities, and antioxidant action. We screened water-soluble fractions because they are easy to include in a wide variety of assays.

All tested extracts of vegetables and their cultivars had similarly effective antioxidant activities, indicating that vitamin C and tannins are the sources of these activities. Particular vegetables contain high antioxidant polyphenols and abundant amounts of tannins [11], but we did not find particularly high antioxidant activity in any tested cultivars that supposedly possess such activity.

Maintaining moderate blood sugar levels is an effective approach to diabetes control, and α-glucosidase is essential for carbohydrate digestion in the intestine for glucose absorption. The inhibition of α-glucosidase activity suppresses the increase in postprandial blood glucose by suppressing glucose absorption [12]. Therefore, α-glucosidase inhibitors have been applied as therapeutic agents for type 2 diabetes mellitus. However, α-glucosidase inhibition by agents such as acarbose and voglibose is unsatisfactory for diabetes management and prolonged use often causes side effects, including diarrhea [13] with concomitant intestinal pain and flatulence [14]. Tannins might be the main inhibitors of α-glucosidase activity in these cultivars [15, 16].

The suppression of triglyceride absorption is directly associated with the prevention of obesity and related diseases. Secreted pancreatic lipase

**Table 5.** Effects of vegetable extract on platelet aggregation, PT, and APTT.

| Vegetable | Cultivar | Platelet aggregation rate (%) | PT (seconds) | APTT (seconds) |
|---|---|---|---|---|
| Control | | 98.5 ± 2.7 | 353.3 ± 7.0 | 256.0 ± 18.4 |
| Cabbage | Kinkei No.201 | 98.6 ± 3.5 | 344.2 ± 5.1 | 256.0 ± 2.0 |
| Onion | Shōnan red | 99.5 ± 1.9 | 353.4 ± 7.3 | 265.1 ± 17.9 |
| Eggplant | Senryo No.2 | 91.5 ± 8.5 | 349.3 ± 5.1 | 249.6 ± 15.0 |
| Tomato | Momotaro | 100.2 ± 2.5 | 352.8 ± 6.1 | 254.5 ± 15.8 |
| Green pepper | Kyōsuzu | 98.9 ± 1.5 | 352.8 ± 2.4 | 251.4 ± 14.1 |
| Radish | Taibyōsōbutori | 97.8 ± 0.8 | 359.6 ± 5.6 | 252.8 ± 16.4 |
| Chinese cabbage | Haregi | 106.1 ± 2.7 | 348.7 ± 9.7 | 259.5 ± 17.5 |

Control and extracts do not significantly differ ($p > 0.05$).

hydrolyzes triglycerides and it is a key enzyme for triglyceride absorption in the small intestine. Therefore, lipase inhibition is a major approach to obesity prevention. Anti-obesity drugs are associated with serious side effects such as bloating, dyspepsia, and diarrhea. Lipase inhibitors derived from natural plants are required for obesity treatment without these side effects [17]. Fabroni et al. recently described the chemical composition and pancreatic lipase inhibitory activities *in vitro* of 13 lyophilized extracts of various Mediterranean plants containing anthocyanins [18]. Platelets play a major role in blood clotting and their aggregation is a crucial step in the process of hemostasis. Excessive platelet aggregation can contribute to life-threatening thrombotic disorders such as stroke and cardiovascular diseases. Therefore, inhibiting platelet aggregation should be an effective approach to help prevent these types of diseases. Natural compounds such as flavonoids derived from plants and other foods can inhibit platelet aggregation [19].

Clinical PT and APTT tests determine how long blood takes to clot. The extrinsic and common pathways of blood coagulation are evaluated in PT assays [20] that reflect the amounts and activities of blood coagulation factors involved in these pathways such as Factor VII, fibrinogen, prothrombin, and Factor X. The APTT assay evaluates the intrinsic pathway and common pathways by reflecting the amounts and activities of Factors V, VIII, IX, X, XI, XII, fibrinogen, and prothrombin.

All tested vegetables and cultivars had antioxidant activities and varying amounts of α-glucosidase and lipase inhibitory activities. However, none of the tested vegetable extracts inhibited platelet aggregation, or affected intrinsic and extrinsic blood coagulation. Many extracts have antioxidant activities, but few have activities that affect other processes**.** All tested vegetables and their cultivars had similarly effective antioxidant activities. Compounds such as vitamin C and tannins that are abundant in vegetables are the sources of these activities.

## Conclusion

The present study screened various vegetable cultivars in Japan for hitherto unknown food functionality that might impact lifestyle diseases and metabolic syndrome. Although we found some functionality in these vegetables, the active components were not clear and the activity was weak. Therefore, these extracts should be analyzed in detail in future studies. Regardless, the present findings provide fundamental information about vegetables consumed as functional foods in Japan. Further investigations are required to determine whether or not the findings of such screens will help to develop new functional foods based on concentrated vegetable extracts.

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