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RESEARCH ARTICLE

Potentials of representative heirloom vegetables on Shonai region of Yamagata, Japan

Takeshi Nagai[1,2,3]*, Takumi Nagata[4,5], Yasuhiro Tanoue[6], Norihisa Kai[7], Nobutaka Suzuki[8]

[1]Graduate School of Agricultural Sciences, Yamagata University, Yamagata 9978555, Japan, [2]The United Graduate School of Agricultural Sciences, Iwate University, Iwate 0208550, Japan, [3]Graduate School, Prince of Songkla University, Songkhla 90112, Thailand, [4]Yamagata University, Yamagata 9978555, Japan, [5]Yataro Group, Shizuoka 4350046, Japan, [6]National Fisheries University, Yamaguchi 7596595, Japan, [7]Oita University, Oita 8701192, Japan, [8]Nagoya Research Institute, Aichi 4701131, Japan

ABSTRACT

The aim of this work was to elucidate the proximate composition, functional components, and functional properties of representative heirloom vegetables on Shonai region of Yamagata, Japan. Turnip roots such as *Fujisawakabu* and *Tomoefuki* contained a lot of proteins and carbohydrates among these vegetables tested. Many vegetables showed about 2-3 times as many vitamin C and β-carotene values as corresponding commercially available vegetables. Overall, water and methanol extracts prepared from these vegetables possessed remarkably high antioxidative activities except for *Makomodake*. Radical scavenging through different mechanisms and hyaluronidase inhibitory activities varied markedly among these vegetables. Particularly, all vegetables exhibited outstanding ACE inhibitory activities about 45.1-95.8%. These findings demonstrated that heirloom vegetables used in this study served as good sources of vitamins, phenolics, and antioxidants compared to corresponding commercially available vegetables. Positively eating of these vegetables can probably contribute to health promotion to prevent life style-related diseases such as cancer, hypertension, and inflammation. Furthermore, it also may have potentials for preservation of species and for promotion of sustainable cultivation of heirloom vegetables.

Keywords: Functional property; Heirloom vegetable; Nutrition; Proximate composition

INTRODUCTION

Vegetables plays important roles as not only resources for supplying micronutrients such as vitamins and minerals that are essential for normal nutrition and metabolisms but also as low-caloric and low-fatty foods. In addition, many kinds of functional ingredients contain in it. Phenolics have multiple biological functions such as antioxidant and antibacterial properties (Fawole et al., 2012) and synergistic effects and protective properties against life style-related diseases such as arteriosclerosis, arthritis, brain dysfunction, cardiovascular diseases, cancer, hypertension, and inflammation (Verma et al., 2018). Reactive oxygen species as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals, which cause oxidative damage of human body, are factors of onset of many diseases containing cancer. Therefore, consumption of vegetables, which are rich in fibers, minerals, phenolics, and vitamins, help to prevent oxidative stress and to reduce incidence of these diseases (Fidrianny et al., 2018). Moreover, it is reported that intake of antioxidants such as phenolics and vitamins retard ageing (Ross and Kasum, 2002).

Heirloom vegetables are crops that seeds are maintained and conservation of species is continued by growers in limited areas over generations. It is considered that these species have values as cultural properties because these are genetic resources for breeding materials and are in close relations with traditional eating habits in a region. However, traditional varieties rapidly disappear worldwide, therefore, it has been discussing the way of conservation of varieties (Tomiyoshi and Ueno, 2016). Recently, there is a nationwide trend to revalue existence and benefits of heirloom vegetables. Representative heirloom vegetables in Japan are as follows: *Shishigatani* pumpkin, *Kujonegi*, and *Fushimi* pepper in Kyoto (JA-Kyoto), *Aizumaru* eggplant, *Arakudakukitachi*, and *Tachikawa* burdock in Aizu region of Fukushima (Association to protect traditional vegetables in Aizu; Mizuno and Sumino, 2008), and *Nakajimana*, *Futatsukakarashina*, and *Kaga* Glycine soja in Kanazawa

***Corresponding author:**
Takeshi Nagai,Graduate School of Agricultural Sciences, Yamagata University, Yamagata 9978555, Japan.
E-mail: nagatakenagatake@yahoo.co.jp, tnagai@tds1.tr.yamagata-u.ac.jp

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region of Ishikawa (Kanazawa City Agricultural Products Branding Association). Particularly, there are many types of heirloom vegetables in Yamagata, Japan (179 varieties in 2018). Among them, 87 varieties of vegetables are cultivated in Shonai region of Yamagata, Japan (Tsuruoka Creative City of Gastronomy Promotion Committee, 2018). However, few studies have explored even proximate composition and nutritional properties of these vegetables. It is expected that many vegetables are more nutritious than commonly available vegetables, as these has not undergo selective breeding aimed at good appearance and convenience for broad area distribution. Consumers nowadays tend to require foods with high nutritional values and health functionalities. This work was performed to evaluate proximate composition and physicochemical and nutritional properties of main heirloom vegetables on Shonai region of Yamagata, Japan, and furthermore to elucidate health functionalities of these vegetables.

MATERIALS AND METHODS

Materials

Fourteen fresh heirloom vegetables (*Atsumikabu*, *Chijimina*, *Fujisawakabu*, *Hirataakanegi*, *Karatori*, *Kirariboshi*, *Makomodake*, *Mindennasu*, *Mosodake*, *Natsuna*, *Okitanasu*, *Tagawakabu*, *Tomoebuki*, and *Tonojimakyuri*) were purchased from produce stands on Shonai region of Yamagata, Japan (Fig. 1), and were used in the study. These are representative heirloom vegetables on this region. In addition, these are eaten frequently on the region. The information about these genetic materials is summarized in the book (Yamagata Forum for the Indigenous Crops, 2012). All chemicals were of regent grade.

Fig 1. Heirloom vegetables on Shonai region of Yamagata, Japan used in the study. (a) *Atsumikabu*, (b) *Tagawakabu*, (c) *Fujisawakabu*, (d) *Okitanasu*, (e) *Mindensasu*, (f) *Karatori* (tubers), (g) *Karatori* (stems), (h) *Kirariboshi*, (i) *Chijimina*, (j) *Natsuna*, (k) *Tonojimakyuri*, (l) *Tomoefuki*, (m) *Hirataakanegi*, (n) *Makomodake*, (o) *Mosodake.*

Determination of proximate composition

Moisture was measured using a Moisture Determination Balance (FD-600; Kett Electric Laboratory, Tokyo, Japan). Crude proteins were determined by the Kjeldahl method using a conversion factor of 6.25. Crude lipids were analyzed by ether extraction. Measurements of crude ashes were used an electric furnace (AMI-II; Nitto Kagaku Co. Ltd., Aichi, Japan). Carbohydrates were calculated by difference. Crude fibers were measured as described by Nakamura et al. (1998). Salts were determined using a digital salinity concentration meter (EB-158P, EISHIN Co., Ltd., Hiroshima, Japan). Calories were calculated using the FAO conversion factors (Isobe et al., 2011).

Physicochemical properties

Total soluble solids (TSS) were determined using a digital refractometer (PAL-Pâtissier, Atago Co. Ltd., Tokyo, Japan). The pH values were measured using a digital pH meter (PHL-40, DKK-TOA Co., Tokyo, Japan). Alkalinity was estimated as described by Miura et al. (2006). Free amino acid contents were determined by the TNBS method (Sugawara and Soejima, 1996) using L-leucine as standard. Vitamins B1 and B2 were measured by the *p*-aminoacetophenone method and by the lumiflavin fluorescence method, respectively (Nakamura et al., 1998). Vitamin C was determined by α,α'-dipyridyl method (The Vitamin Society of Japan, 1990). β-Carotene, lycopene, and chlorophyll (a, b) were measured using acetone-hexane extraction method (Nagata and Yamashita, 1992). Phenols and flavonoids were determined as described by Slinkard and Singleton (1977) and by Kim et al. (2003), respectively, using quercetin dihydrate as standard.

Functional properties

Antioxidative activities of extracts from vegetables were evaluated as described by Nagai et al. (2018). Ascorbic acid (AA), *tert*-butyl-4-hydroxyanisole (BHA), 2,6-di-*t*-butyl-4-methylphenol (BHT), α-tocopherol (TP), and trolox (TL) were used as positive controls, and distilled water or 80% methanol were used as negative one. Superoxide anion radicals, hydroxyl radicals, and DPPH radicals scavenging activities were determined as described by Nagai et al. (2018). Activities [TL equivalents scavenging capacity

(TESC); mM TE/kg FW] were also expressed as millimoles of TL equivalents per kg of fresh weight of vegetables. ACE and hyaluronidase inhibitory activities were measured as described by Nagai et al. (2018).

Statistical analysis

Each assay was repeated 3 times independently and results were reported as means ± standard deviation (SD).

RESULTS AND DISCUSSION

Proximate composition

Proximate composition per 100g of vegetables is shown in Table 1. Moisture contents ranged from 90.1-96.0 g except for *Karatori* tubers and *Chijimina*. Content in *Karatori* tubers was similar to eddoe fresh bulbs, but was fairly lower than taro fresh bulbs (Kagawa, 2018). Protein contents were high in *Kirariboshi* about 3.4 g, followed by *Chijimina*, *Fujisawakabu*, and *Mosodake*, whereas *Karatori* stems were lowest. Turnip roots showed higher content than commercially available turnip (fresh roots with skin). Lipids were not detected at all or were low about 0.1 g except for *Kirariboshi*, *Chijimina*, and *Natsuna*. *Karatori* tubers contained large amount of carbohydrates, suggesting existence of great quantities of starches. Fiber contents were high in leafy vegetables such as *Kirariboshi*, *Chijimina*, and *Natsuna*. Ash contents ranged from 0.4-1.2 g. Salts were not mostly detected in these vegetables. Highest energy was calculated in *Karatori* tubers about 95.8 kcal, which carbohydrate content was highest among these vegetables, followed by in *Tomoefuki*, *Hirataakanegi*, *Kirariboshi*, and *Mosodake*.

Physicochemical properties

Vegetables were cut into small pieces, ground in a mortar, and then TSS and pH at 20ºC were measured. Highest TSS value was detected in *Chijimina*, followed by in *Hirataakanegi*, *Atsumikabu*, and *Okitanasu*, whereas *Karatori* tubers were lowest because of existence of great quantities of starches (Table 1). Correlation between carbohydrate contents and TSS contents was with R^2 = 0.648 except for *Karatori* tubers and *Tomoefuki* and was with R^2 = 0.833 except for *Fujisawakabu*. The pH values ranged from 5.4-6.4. High alkarinity was shown in *Chijimina*, followed by *Makomodake*, *Kirariboshi*, and *Fujisawakabu*, whereas *Tonojimakyuri*, *Mindennasu*, and *Okitanasu* were low. There were varietal differences on alkarinity among turnip roots and leafy vegetables. Generally, grains, meats, and fish and shellfishes are classified as acid foods, whereas vegetables, fruits, mushrooms, and seaweeds are categorized as alkaline ones. Highest free amino acid contents were shown in *Mosodake*, followed by in *Chijimina*, *Kirariboshi*, and *Makomodake*, whereas *Karatori* stems were low.

Functional components

Kirariboshi showed high vitamin B1 value, followed by *Chijimina* and *Natsuna*, whereas turnip roots as *Fujisawakabu*, *Karatori* tubers, *Tomoefuki*, and *Makomodake* were low (Table 1). Vitamin B2 contents were highest in *Chijimina* about 0.15 mg, followed by in *Natsuna*, *Kirariboshi*, and *Mindennasu*. *Karatori* (stems and tubers) and *Tonojimakyuri* were lowest. *Chijimina* showed more than two times as high content as green pak choi fresh leaves. *Tagawakabu* possessed high vitamin C about 61.9 mg, followed by *Fujisawakabu*, *Kirariboshi*, *Atsumikabu*, and *Chijimina*, whereas *Makomodake* and *Tomoefuki* were lowest. Contents on turnip roots as *Atsumikabu* were about 2.2-3.3 times as high as commercially available turnip. *Okitanasu* and *Mindennasu* showed vitamin C about 2.5-3.3 times as high as eggplant and *Beinasu*. Yamaguchi et al. (2012) measured ascorbic acid contents in 12 traditional vegetables (*Yamatoyasai*) of Nara, Japan. *Himotogarashi* exhibited high value about 28.0 mg/100 g FW, followed by *Murasakitogarashi*, *Yamatonama*, and *Chisujimizuna* (about 22.4-25.8 mg/100 g FW). Except for *Karatori*, β-carotene contents were significantly higher than those on commercially available vegetables. Lycopene was not detected in all vegetables. Chlorophyll a and b showed high values in leafy vegetables such as *Kirariboshi* and *Natsuna*.

Next, vegetables were cut into small pieces, and were homogenized with 2 volumes of distilled water or 80% methanol. Suspension was centrifuged at 30,000 x g for 30 min at 4ºC, and supernatants were filtered with glass wool (distilled water extracts: WE; methanol extracts: ME). Phenols and flavonoids contents of these extracts are shown in Table 1. In WEs, *Chijimina*, *Kirariboshi*, and *Tomoefuki* showed high phenols values about 175.9, 147.0, and 137.1 mg, respectively, followed by *Mosodake* and *Okitanasu*. *Fujisawakabu* and *Makomodake* were low. In MEs, *Tomoefuki* was highest about 361.4 mg, followed by *Chijimina*, *Kirariboshi*, and *Mindennasu*, whereas *Tonojimakyuri* and *Atsumikabu* were low. These are supported by the findings that butterburs contain a large amount of phenol compounds such as chlorogenic acid, kaempferol, quercetin, fukinolic acid, and fukinone. In WEs, *Kirariboshi* and *Chijimina* showed highest flavonoids values about 145.5 and 141.7 mg, respectively, followed by *Tomoefuki*. *Hirataakanegi*, *Makomodake*, and *Tonojimakyuri*. Positive correlation was observed with R^2 = 0.753 between phenols and flavonoids contents in WEs (R^2 = 0.938 except for *Mosodake*). Flavonoids contents accounted for 90.1-99.0% to phenols of WEs (*Kirariboshi*, *Mindennasu*, and *Karatori* stems and tubers): most of polyphenols was accounted for flavonoids. Meanwhile, its rates were low in *Mosodake* (13.9%), *Hirataakanegi* (20.7%), *Makomodake* (30.0%), and *Tonojimakyuri* (33.1%), suggesting existence of large quantities of phenolic components except for

Table 1: Physicochemical properties of heirloom vegetables on Shonai region of Yamagata, Japan

Property	*Atsumikabu*	*Tagawakabu*	*Fujisawakabu*	*Okitanasu*	*Mindennasu*	*Karatori* (tubers)	*Karatori* (stems)	*Kirariboshi*
Energy (kcal)	23.4	18.1	16.7	22.3	21.5	95.8	14.2	27.3
Water (g/100g)	93.1±0.3	94.6±0.3	94.6±0.2	92.7±0.1	92.7±0.5	73.9±0.2	95.1±0.2	90.1±0.2
Protein (g/100g)	1.1±0.1	1.0±0.1	2.4±0.2	1.8±0.1	1.9±0.2	1.2±0.1	0.4	3.4±0.2
Lipid (g/100g)	0.1	0.1	0	0.1	0.1	0.1	0.1	0.3±0.1
Carbohydrate (g/100g)	5.2	3.9	2.6	4.9	4.6	23.9	3.6	5.0
Fiber (g/100g)	2.5±0.4	2.6±0.2	2.2±0.1	1.9	2.1±0.1	2.0	1.8±0.1	3.3±0.1
Ash (g/100g)	0.5	0.4	0.4	0.5	0.7	0.8	0.8	1.2
Salt equivalents (g/100g)	0	0	0.1	0	0	0	0.1	0
Vitamin B_1 (mg/100g)	0.03±0.01	0.03±0.01	0.02±0.01	0.02	0.07	0.03	0.07	0.10
Vitamin B_2 (mg/100g)	0.05	0.04	0.05	0.05	0.07	0.01	0.01	0.07
Vitamin C (mg/100g)	42.5±0.3	61.9±1.1	54.3±0.1	19.8±0.4	14.9±0.2	8.1±0.1	21.6±0.1	43.2±0.7
β-Carotene (μg/100g)	7.0±4.9	0	0	154.0±1.5	121.0±1.2	138.0±1.1	55.0±4.9	6164.0±861
Lycopene (mg/100g)	0	0	0	0	0	0	0	0
Chlorophyll a (mg/100g)	0	0	0	0.2±0.1	0.3±0.1	0	0.3±0.1	13.3±1.3
Chlorophyll b (mg/100g)	0	0	0	0	0.1	0	0.2	5.9±0.4
Free amino acids (mg/100g)	56.1±0.4	90.2±0.2	67.7±0.5	115.9±1.3	72.5±0.5	47.4±1.5	12.6±0.2	173.2±1.2
Total phenols (mg/100g) WEs	43.8±1.2	84.8±1.5	25.7±4.5	93.0±5.8	88.1±4.1	61.8±3.9	52.0±2.7	147.0±3.1
Total phenols (mg/100g) MEs	40.9±2.3	67.6±1.9	42.3±2.4	82.7±4.7	101.7±4.5	64.2±5.9	57.4±4.3	115.8±2.6
Total flavonoids (mg/100g) WEs	27.0±3.5	75.5±7.4	14.8±2.9	77.1±12.7	84.2±10.5	55.7±6.7	48.5±9.9	145.5±3.1
Total flavonoids (mg/100g) MEs	25.6±5.1	66.3±2.7	16.3±2.2	77.1±6.8	62.8±5.5	41.4±7.4	34.3±6.3	96.2±3.0
TSS (%)	5.9±0.1	4.6±0.1	4.9±0.1	5.8	4.9	1.8±0.1	3.5	5.0±0.2
pH	5.8	5.4	5.8	5.7	5.5	6.2	5.8	6.3
Alkarinity	9.2±1.0	15.0±0.8	18.0±0.1	5.8±0.1	5.5±0.2	8.6±0.2	11.6±0.6	18.3±5.8
Property	***Chijimina***	***Natsuna***	***Tonojimakyuri***	***Tomoefuki***	***Hirataakanegi***	***Makomodake***	***Mosodake***	
Energy (kcal)	31.5	17.1	11.7	28.3	28.2	22.6	26.4	
Water (g/100g)	89.5±0.3	93.9±0.2	96.0±0.3	91.2±0.3	91.2±0.5	93.3±0.2	92.1±0.2	
Protein (g/100g)	2.8±0.2	1.1±0.1	0.7±0.1	0.9±0.1	1.5±0.1	1.2±0.2	2.3±0.1	
Lipid (g/100g)	0.2	0.4	0.1	0.1	0.1	0.1	0.1	
Carbohydrate (g/100g)	6.7	3.6	2.7	7.2	6.7	4.9	5.1	
Fiber (g/100g)	3.6±0.1	3.2±0.1	1.2	0.8	0.4±0.1	1.8	2.2±0.1	
Ash (g/100g)	0.8	1.0	0.5	0.6	0.6	0.4	0.5	
Salt equivalents (g/100g)	0	0.1	0	0.1	0	0	0	
Vitamin B_1 (mg/100g)	0.09	0.08	0.05	0.02±0.01	0.04	0.03±0.01	0.04±0.01	
Vitamin B_2 (mg/100g)	0.15	0.09±0.01	0.02	0.05±0.01	0.03	0.03	0.06	
Vitamin C (mg/100g)	41.6±0.8	12.8±0.5	5.7	4.0±0.4	20.4±0.6	3.2±0.3	20.7±0.6	
β-Carotene (μg/100g)	7075.0±675	5757.0±737	435.9±139	454.0±81	1358.0±438	77.0±3.5	308.0±8.1	
Lycopene (mg/100g)	0	0	0	0	0	0	0	
Chlorophyll a (mg/100g)	12.5±0.6	13.1±0.9	1.3±0.1	1.4	3.9±0.6	0	0	
Chlorophyll b (mg/100g)	4.8±0.2	5.9±0.3	0.7	0.6	1.5±0.2	0	0	

(*Contd...*)

Table 1: (*Continued*)

Property	Chijimina	Natsuna	Tonojimakyuri	Tomoefuki	Hirataakanegi	Makomodake	Mosodake
Free amino acids (mg/100g)	260.6±17.7	87.3±0.7	77.7±2.3	28.2±0.4	25.7±2.5	134.4±4.0	556.4±2.2
Total phenols (mg/100g) WEs	175.9±8.9	57.2±4.4	34.1±2.3	137.1±4.9	44.9±3.5	35.3±2.1	112.5±19.7
Total phenols (mg/100g) MEs	118.1±2.4	61.6±1.8	36.7±2.0	361.4±1.6	46.1±4.4	48.9±3.0	78.9±7.1
Total flavonoids (mg/100g) WEs	141.7±5.6	48.4±3.9	11.3±2.4	105.7±7.3	9.1±1.9	10.6±1.5	15.6±2.0
Total flavonoids (mg/100g) MEs	111.2±3.7	61.3±2.9	3.4±1.2	177.1±15.6	4.1±1.2	7.0±1.0	19.1±0.9
TSS (%)	6.5±0.1	2.8±0.1	3.0	3.3±0.2	6.2	4.8±0.1	5.3±0.4
pH	6.4	6.1±0.1	5.7	6.0	5.6	5.6	6.0
Alkarinity	26.3±4.5	6.7±3.2	2.5±0.2	12.3±1.2	14.5±1.6	18.5±0.8	11.3±1.2

flavonoids. In MEs, *Tomoefuki* showed highest flavonoid value, followed by *Chijimina* and *Kirariboshi*, whereas *Tonojimakyuri*, *Hirataakanegi*, and *Makomodake* were low. Positive correlation with $R^2 = 0.761$ was observed between phenols and flavonoids contents. The rates of flavonoids to phenols were significantly high in *Natsuna*, *Tagawakabu*, *Chijimina*, and *Okitanasu* (93.2-99.5%). Cultivars, pre-harvest climate conditions, and maturities of vegetables can be defined as crucial factors affecting these functional components biosynthesis and accumulation. Yamaguchi et al. (2012) reported phenol contents in 12 *Yamatoyasai* vegetables: *Yamatonama* showed highest value about 5.9 mg gallic acid equivalent (GAE)/kg FW, followed by *Kaorigobo* and *Udakingobo* (5.4 mg GAE/kg FW), whereas *Hanshirokyuri* and *Yamatosanzyakukyuri* were low about 0.8-1.0 mg GAE/kg FW. Itou et al. (2010) investigated polyphenol contents of 12 root vegetables in Ehime, Japan. *Yamagobo* contained high polyphenols [2.3 g chlorogenic acid equivalent (CAE)/kg FW], followed by lotus root (2.1 g CAE/kg FW) and *Shodaikon* (1.0 g CAE/kg FW). Jersey cudweed (*Gogyo*) showed significantly high content (8.1 g CAE/kg FW) compared to other leafy vegetables such as common henbit and shepherd's purse. Moreover, *Kinukawanasu* showed moderate value about 3.8 g CAE/kg FW, but loquat *hacha* was lowest about 0.1 g CAE/kg FW.

Antioxidative activity

In WEs, *Okitanasu* showed significantly high antioxidative activity similar to 1.0 mM TL (Table 2). Activities of *Mindennasu*, *Chijimina*, and *Kirariboshi* were higher than those of 5.0 mM AA and 1.0 mM BHT. *Mosodake*, *Karatori* stems, and *Natsuna* showed same activities as 0.1 mM BHA, BHT, and TL, whereas, *Fujisawakabu*, *Makomodake*, and *Tonojimakyuri* were low. MEs from *Kirariboshi* and *Chijimina* possessed highest activities similar to 1.0 mM TP and TL (Table 2). *Natsuna*, *Mindennasu*, and *Okitanasu* showed same activities as 5.0 mM AA and 1.0 mM BHA and BHT. Meanwhile, *Makomodake* was low. Ismail et al. (2004) measured antioxidant activities using β-carotene bleaching system and phenolic contents in cabbage, kale, shallots, spinach, and swamp cabbage. Shallots showed high activity about 69.1%, followed by spinach, swamp cabbage, cabbage, and kale. Meanwhile, phenol content was highest in spinach, followed by in swamp cabbage, kale, shallots, and cabbage. Correlation was not observed between antioxidant activities and phenol contents.

Superoxide anion radical scavenging activity

In WEs, superoxide anion radical scavenging activity on *Tomoefuki* was highest about 90.4%, followed by *Makomodake*, *Natsuna*, *Atsumikabu*, and *Mindennasu*, whereas *Karatori* stems were low about 32.3% (Table 3). TESC was estimated to 0.11-0.33 x 10^3 mM TE/kg FW. In MEs, *Tagawakabu* possessed high activity about 88.7%,

Table 2: Antioxidative activities of WEs and MEs from heirloom vegetables on Shonai region of Yamagata, Japan

Samples	Absorbance (500 nm)					
	50 min		100 min		200 min	
	WEs	MEs	WEs	MEs	WEs	MEs
Atsumikabu	0.034±0.003	0.006±0.001	0.102±0.001	0.062±0.003	0.287±0.001	0.069±0.004
Tagawakabu	0.057±0.001	0.025±0.002	0.128±0.002	0.042	0.253±0.003	0.163±0.004
Fujisawakabu	0.085±0.002	0.008±0.002	0.159	0.027±0.002	0.389±0.006	0.058±0.002
Okitanasu	0.013±0.006	0.049±0.002	0.028±0.009	0.050±0.005	0.037±0.008	0.091±0.010
Mindennasu	0.039±0.005	0.047±0.007	0.049±0.014	0.075±0.009	0.056±0.003	0.098±0.008
Karatori (tubers)	0	0.103±0.008	0.141±0.005	0.138±0.006	0.189±0.007	0.284±0.012
Karatori (stems)	0.056±0.003	0.178±0.020	0.105±0.008	0.157±0.002	0.148±0.004	0.206±0.006
Kirariboshi	0.036	0.003±0.002	0.031	0	0.064±0.003	0.042±0.001
Chijimina	0.023±0.002	0.014±0.002	0.018	0	0.058±0.003	0.043±0.002
Natsuna	0.019±0.002	0.097±0.027	0.015±0.003	0.050±0.006	0.168±0.001	0.101±0.002
Tonojimakyuri	0.135±0.003	0.116±0.013	0.274±0.006	0.189±0.008	0.418±0.003	0.286±0.011
Tomoefuki	0.040	0.029±0.003	0.061	0.050±0.007	0.250±0.002	0.134±0.002
Hirataakanegi	0.152±0.005	0.078±0.002	0.261±0.014	0.412±0.006	0.367±0.011	0.273±0.012
Makomodake	0.113±0.006	0.103±0.021	0.187±0.001	0.196±0.001	0.395±0.006	0.429±0.003
Mosodake	0.040±0.001	0.030	0.103	0.071	0.147±0.001	0.148±0.003
1.0 mM AA	0.022±0.001		0.135±0.006		0.469±0.027	
5.0 mM AA	0.016±0.001		0.032±0.003		0.090±0.008	
0.01 mM BHA	0.084±0.005		0.120±0.008		0.245±0.012	
0.1 mM BHA	0.056±0.003		0.090±0.006		0.165±0.010	
1.0 mM BHA	0.054±0.002		0.057±0.003		0.100±0.006	
0.01 mM BHT	0.082±0.003		0.112±0.009		0.248±0.011	
0.1 mM BHT	0.058±0.004		0.108±0.005		0.173±0.008	
1.0 mM BHT	0.044±0.002		0.051±0.003		0.093±0.005	
1.0 mM TP	0.006		0.025±0.001		0.028±0.002	
0.01 mM TL	0.084±0.005		0.094±0.006		0.262±0.013	
0.1 mM TL	0.038±0.002		0.051±0.003		0.123±0.008	
1.0 mM TL	0.011±0.001		0.031±0.002		0.032±0.002	
Control	0.379±0.008		0.715±0.025		1.406±0.041	

followed by *Atsumikabu* and *Tomoefuki*. Meanwhile, *Karatori* stems and tubers were low. TESC ranged from 0.04-0.32 x 10^3 mM TE/kg FW. Correlation was not observed between activities on WEs or MEs and phenols or flavonoids contents. Kimura et al. (2002) reported that green tea possessed highest activity (197.2 U/g DW) among on 45 vegetables, while perilla was low about 20.0 U/g dry weight (U/g DW). Hirata (2010) investigated radical scavenging activities of 43 vegetables in Yamagata, Japan. High activity (IC50 = 0.0026 g/ml extract) was detected in eggplant (*Tayanasu*), whereas *Chikuyo* showed no activity. Onion (*Yamaguchikodaka*), bitter melon, and tomato (*Momotaro*) possessed high activities with IC50 = 0.004, 0.0042, and 0.005 g/ml extract, respectively. Meanwhile, leaf mustards (*Hikoshimaharuna*, *Miike*) was low (IC50 = 0.0252 g/ml extract). Turnip (*Hagikoroge*, *Takehisa*, and *Suwan*), bok choy, Chinese mustard, and rapeseed (*Hanakkori*) did not detect the activities.

Hydroxyl radical scavenging activity

In WEs, *Kirariboshi* and *Karatori* stems showed remarkably high hydroxyl radical scavenging activities about 85.7 and 85.3%, respectively, although activities did not reach that of 0.1 mM TL (Table 3). Activity on *Natsuna* was similar to that in 0.01 mM BHT. On the contrary, *Tonojimakyuri* and *Mindennasu* were low. TESC ranged from 0.13-1.54 x 10^3 mM TE/kg FW. In MEs, *Tomoefuki* exhibited significantly high activity about 88.2%, followed by *Karatori* stems, whereas *Mosodake* was low about 34.3%. TESC ranged from 0.39-1.59 x 10^3 mM TE/kg FW. Correlation was not observed between activities on WEs or MEs and phenols or flavonoids contents.

DPPH radical scavenging activity

In WEs, *Tomoefuki* and *Kirariboshi* possessed highest DPPH radical scavenging activities about 85.9 and 84.5%, respectively, whereas *Makomodake* and *Mosodake* were low (Table 3). Particularly, *Tonojimakyuri* was lowest. TESC ranged from 26.4-150.6 x 10^3 mM TE/kg FW. In MEs, activities of *Tagawakabu* and *Fujisawakabu* were significantly high (86.6 and 86.3%, respectively). *Tonojimakyuri* was lowest among these vegetables. TESC ranged from 23.9-152.0 x 10^3 mM TE/kg FW. DPPH radical scavenging activity is correlated with phenol contents (Velioglu et al., 1998). However, correlation was not observed between activities of WEs or MEs and phenols or flavonoids contents. Yamaguchi et al. (2012) investigated DPPH radical

Table 3: Superoxide anion radicals, hydroxyl radicals, and DPPH radicals scavenging activities of WEs and MEs from heirloom vegetables on Shonai region of Yamagata, Japan

Samples	Scavenging activity (%)					
	Superoxide anion radicals		Hydroxyl radicals		DPPH radicals	
	WEs	MEs	WEs	MEs	WEs	MEs
Atsumikabu	82.3±3.9 (0.30)	76.0±2.1 (0.27)	46.2±5.3 (0.66)	52.9±5.8 (0.80)	76.3±1.5 (130.8)	78.8±0.9 (136.0)
Tagawakabu	71.6±4.2 (0.26)	88.7±3.0 (0.32)	47.8±4.9 (0.69)	76.5±5.9 (1.33)	72.6±1.7 (123.2)	86.6±1.1 (152.0)
Fujisawakabu	77.8±3.5 (0.28)	74.1±5.2 (0.27)	52.9±6.7 (0.80)	54.4±6.0 (0.84)	74.7±2.0 (127.6)	86.3±1.9 (151.5)
Okitanasu	65.6±4.2 (0.23)	60.8±1.9 (0.22)	62.2±6.4 (1.01)	73.3±4.7 (1.26)	82.9±1.1 (144.4)	79.9±1.5 (138.3)
Mindennasu	81.9±3.8 (0.29)	57.5±5.7 (0.20)	24.4±4.1 (0.17)	74.2±6.0 (1.28)	28.8±2.9 (33.0)	60.7±2.2 (98.7)
Karatori (tubers)	50.2±3.3 (0.18)	12.9±5.1 (0.04)	61.0±8.2 (0.99)	62.1±4.5 (1.01)	70.1±1.5 (118.1)	76.1±1.8 (130.4)
Karatori (stems)	32.3±6.9 (0.11)	10.7±2.8 (0.03)	85.3±3.9 (1.53)	81.3±2.1 (1.44)	55.1±1.4 (87.2)	55.6±2.4 (88.2)
Kirariboshi	70.0±4.7 (0.25)	33.8±14.1 (0.11)	85.7±2.8 (1.54)	73.5±2.8 (1.27)	84.5±1.8 (147.7)	83.5±1.9 (145.7)
Chijimina	60.7±5.4 (0.22)	41.2±10.2 (0.14)	74.8±8.8 (1.29)	70.9±3.4 (1.21)	73.7±2.7 (125.5)	78.2±2.0 (134.8)
Natsuna	83.4±8.5 (0.30)	48.5±12.1 (0.17)	82.1±6.9 (1.46)	66.3±4.6 (1.10)	80.3±1.1 (139.1)	79.4±2.3 (137.2)
Tonojimakyuri	64.7±3.8 (0.23)	38.5±4.9 (0.13)	22.7±3.3 (0.13)	53.2±7.1 (0.81)	10.3±2.7 (nd)	12.8±2.7 (nd)
Tomoefuki	90.4±2.1 (0.33)	74.6±2.1 (0.27)	42.2±5.0 (0.57)	88.2±4.2 (1.59)	85.9±1.2 (150.6)	68.9±1.8 (115.6)
Hirataakanegi	56.0±4.5 (0.20)	37.0±4.3 (0.13)	78.8±7.8 (1.38)	65.6±5.8 (1.09)	80.0±0.9 (138.5)	76.4±2.0 (131.1)
Makomodake	85.8±5.2 (0.31)	68.8±3.6 (0.25)	75.1±4.9 (1.30)	72.9±6.1 (1.25)	25.6±2.8 (26.4)	23.9±2.5 (22.9)
Mosodake	70.5±4.6 (0.25)	53.7±3.5 (0.19)	72.1±9.7 (1.23)	34.3±5.9 (0.39)	28.8±4.1 (33.0)	29.3±2.8 (34.0)
1.0 mM AA	14.7±0.2		13.2±0.2		3.1*	
5.0 mM AA	89.9±5.3		17.6±0.7		34.1±2.0**	
0.01 mM BHA	29.3±0.5		59.1±0.8		5.5	
0.1 mM BHA	36.4±0.9		93.3±1.4		17.5±0.4	
1.0 mM BHA	51.9±1.4		95.2±1.4		72.7±3.6	
0.01 mM BHT	11.7±0.2		82.8±0.9		3.9	
0.1 mM BHT	46.6±1.0		97.6±1.6		7.9±0.1	
1.0 mM BHT	48.4±1.2		>100		31.7±0.8	
1.0 mM TP	52.6±4.2		67.6±4.3		87.6±2.8	
0.01 mM TL	46.4±1.0		81.5±0.6		0.1	
0.1 mM TL	58.1±1.1		91.8±1.2		17.9±0.2	
1.0 mM TL	76.1±1.9		>100		86.3±3.3	

*0.1 mM AA; **1.0 mM AA. Values in brackets are 10^3 millimoles of TL equivalents per kg of fresh weight of vegetables.

scavenging activities of *Yamatoyasai* vegetables. *Kaorigobo* showed highest TESC about 37.9 x 10^3 mM TE/kg FW, followed by *Udakingobo* and *Koshoga*, whereas *Hanshirokyuri* and *Yamatosanzyakukyuri* exhibited low TESC (0.35 and 0.34 x 10^3 mM TE/kg FW, respectively). Correlation was shown with R^2 = 0.745 between oxygen radical absorbance capacity and polyphenol contents. Itou et al. (2010) reported that lotus root possessed highest TESC (7.6 mM TE/kg FW), followed by *Shodaikon* (1.9 mM TE/kg FW), whereas elephant foot (tubers) was lowest (0.4 mM TE/kg FW) on local agricultural products in Ehime, Japan. Pea (*Orandaendo*) showed highest TESC about 4.7 mM TE/kg FW, while loquat *hacha* was lowest. Meanwhile, common henbit (20.8 mM TE/kg FW) and Jersey cudweed (*Gogyo*) (15.8 mM TE/kg FW) possessed significantly higher activities than other vegetables. Correlation was not observed between TESC and polyphenol contents. Hirata (2010) reported DPPH radical scavenging activities of agricultural products in Yamaguchi, Japan. TESC was highest in crown daisy (*Oba*) (9.0 mM TE/kg FW) and burdock (*Takinokawa*) (8.7 mM TE/kg FW), but few activities were detected on leaf mustard, bok choy, and Chinese mustard. *Kujonegi* showed middle activity (2.9 mM TE/kg FW).

ACE inhibitory activity

WEs on all vegetables showed high ACE inhibitory activities (Table 4). *Atsumikabu*, *Tagawakabu*, *Okitanasu*, and *Makomodake* possessed high activities of more than 90%, followed by *Mosodake*, *Tonojimakyuri*, *Karatori* stems, and *Mindennasu*. *Natsuna* was middle activity about 52.7%. While, every ME showed high activity of more than 71.1% except for *Tomoefuki*. Particularly, *Fujisawakabu*, *Okitanasu*, *Tonojimakyuri*, and *Mosodake* exhibited significantly high activities about 93.1-95.8%. Correlation with R^2 = 0.621 was observed between activities on MEs and flavonoids contents. Nicotianamine is an ACE inhibitor in mushrooms (Izawa and Aoyagi, 2006). Correlation was shown between its contents on various beans and these activities (Izawa et al., 2008). Izawa and Aoyagi (2012) measured ACE inhibitory activities and nicotianamine contents in 80 vegetables. Surprisingly, asparagus and hosta (*Urui*) showed high activities, although these did not contain nicotianamine. Kimura et al. (2007) investigated ACE inhibitory activities on 7 heirloom vegetables in Yamanashi, Japan. *Mizukakena* (mizuna) showed high activity (IC50 = 8.8 g/100 ml extract), followed by *Mizukakena* (fuyuna). Enomoto (2003) report that *Nakajimana* and

Table 4: ACE and hyaluronidase inhibitory activities of WEs and MEs of heirloom vegetables on Shonai region of Yamagata, Japan

Vegetables	ACE inhibitory activity (%)		Hyaluronidase inhibitory activity (%)	
	WEs	MEs	WEs	MEs
Atsumikabu	90.6±4.1	82.8±3.5	83.8±3.9 (1.43)	43.3±2.1 (0.80)
Tagawakabu	94.5±4.5	89.4±4.7	60.1±4.1 (1.07)	79.1±1.2 (1.35)
Fujisawakabu	65.5±4.7	93.3±3.8	76.5±3.2 (1.32)	48.1±5.9 (0.87)
Okitanasu	95.3±4.2	95.8±5.0	18.9±5.1 (0.42)	88.1±3.7 (1.50)
Mindennasu	84.5±3.8	72.9±1.6	40.4±1.9 (0.75)	51.5±4.1 (0.93)
Karatori (tubers)	72.9±8.5	82.3±3.7	65.5±3.0 (1.14)	87.4±0.8 (1.49)
Karatori (stems)	86.3±4.3	81.8±3.2	81.7±3.1 (1.38)	92.7±1.0 (1.58)
Kirariboshi	75.6±4.0	71.1±5.9	74.5±1.5 (1.29)	84.2±0.6 (1.44)
Chijimina	62.1±18.2	76.1±5.1	38.9±6.1 (0.72)	50.1±1.2 (0.90)
Natsuna	52.7±6.5	80.6±4.2	52.4±4.0 (0.95)	31.9±6.0 (0.62)
Tonojimakyuri	86.6±5.7	93.1±4.4	4.7±2.5 (0.20)	6.2±3.9 (0.21)
Tomoefuki	69.6±10.1	45.1±8.2	32.7±1.2 (0.63)	39.4±6.9 (0.74)
Hirataakanegi	81.2±3.8	83.0±3.6	38.6±4.9 (0.72)	37.9±1.5 (0.72)
Makomodake	90.6±6.5	87.4±2.5	14.0±3.8 (0.33)	57.6±2.6 (1.02)
Mosodake	89.0±4.9	95.7±4.3	16.0±3.5 (0.38)	28.0±5.4 (0.56)

Values in brackets are 10^2 millimoles of sodium cromoglicate equivalents per kg of fresh weight of vegetables.

Futatsukakarashina possessed strongly activities among 12 heirloom vegetables in Ishikawa, Japan.

Hyaluronidase inhibitory activity

Sodium cromoglicate (SC) is used as one of commercially available anti-allergic drug against atopic dermatitis based on food allergies, allergic rhinitis, and allergic conjunctivitis. Hyaluronidase inhibitory activities were also expressed as millimoles of SC equivalents per kg of fresh weight of vegetables [SC equivalents inhibitory capacity (SCEIC); mM SCE/kg FW]. In WEs, *Atsumikabu* and *Karatori* stems showed extremely high activities about 83.3 and 81.7%, respectively, followed by *Fujisawakabu* and *Kirariboshi* (Table 4). Meanwhile, *Okitanasu*, *Makomodake*, and *Mosodake* were low about 14.0-18.9%. SCEIC was estimated to 0.20-1.43 x 10^2 mM SCE/kg FW. In MEs, *Karatori* stems possessed significantly high activity about 92.7%, followed by *Okitanasu*, *Karatori* tubers, and *Kirariboshi*. *Fujisawakabu*, *Mindennasu*, *Chijimina*, and *Makomodake* showed middle activities ranging from 48.1-57.6%, whereas *Tonojimakyuri* was lowest. SCEIC ranged from 0.21-1.58 x 10^2 mM SCE/kg FW. Correlation was not observed between activities and phenols or flavonoids contents. Ippoushi et al. (2000) investigated hyaluronidase inhibitory activities on 46 vegetables and herb extracts. Extracts prepared from 8 *Labiatae* plants and borage showed activities. Moreover, they isolated and identified rosmarinic acid as inhibitor from lemon balm extract. Kimura et al. (2007) reported that activities on 7 heirloom vegetables in Yamanashi, Japan were low or not detected. Hirata (2010) elucidated that none of activities were low, although 32 vegetables exhibited activities among 43 vegetables in Yamaguchi, Japan.

In this study, it was revealed for the first time the nutritional and health-promoting properties of representative heirloom vegetables on Shonai region of Yamagata, Japan. Further research is going to investigate the properties of other vegetables because of existence of many native varieties of vegetables in this area. It is also necessary to research the nutrients and functionalities on each part of vegetable. Cooking may affect the contents of useful components in vegetables. Detailed analysis of nutritional and functional properties on vegetables before and after cooking is in progress to develop novel processing methods of these vegetables.

CONCLUSIONS

These findings demonstrated that heirloom vegetables used in this study served as good sources of vitamins, phenolics, and antioxidants compared to corresponding commercially available vegetables. Positively eating of these vegetables can probably contribute to health promotion to prevent life style-related diseases such as cancer, hypertension, and inflammation. Furthermore, it also may have potentials for preservation of species and for promotion of sustainable cultivation of heirloom vegetables.

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CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

Author contributions

All authors designed the work, acquired, analyzed, interpreted the data, and wrote and revised the manuscript.

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