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RESEARCH

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Taste characteristics, volatile components, sensory properties, and antioxidant activity of fresh onion (*Allium cepa* L.) leaves

Masahiro Yuasa[1,2*], Mayuko Ueno[2], Koji Kawabeta[3,4], Maho Morikawa[3], Momoe Uemura[2], Tetsuhiro Matsuzawa[2] and Mihoko Tominaga[5]

Abstract

Background: To evaluate the usefulness of fresh onion (*Allium cepa* L.) leaves, a food waste, as a new food resource, we characterized the taste characteristics, volatile compounds, sensory properties, and antioxidant activity of fresh onion leaves compared with those of Welsh onion (*Allium fistulosum* L.) (green leaf, small variety) leaves.

Results: The total sugar (g/100 g), total organic acid (mg/100 g), and total free amino acid (mg/100 g) concentrations of fresh onion leaves were 2.12 ± 0.15, 730.02 ± 17.43, and 93.72 ± 7.17, respectively, and were significantly ($p < 0.05$) lower than those of Welsh onion leaves (2.38 ± 0.07, 907.23 ± 20.79, and 131.34 ± 10.22, respectively). In fresh onion leaves, dipropyl disulfide concentration was higher than five times that in Welsh onion leaves. Regarding the sensory properties of the boiled samples, the fresh onion leaves' color was less favorable than Welsh onion leaves ($p < 0.05$), and the taste and aroma were indifferent. The total oxygen radical absorbance capacity value (μmol Trolox equivalents/100 g) and 1,1-diphenyl-2-picrylhydrazyl free radical-scavenging activity (μmol Trolox equivalents/100 g) of fresh onion leaves were 805.78 ± 100.32 and 406.70 ± 63.64, respectively, and differed insignificantly compared with Welsh onion leaves (888.00 ± 112.61 and 382.98 ± 26.08, respectively). However, the total phenolic content (mg gallic acid equivalents/100 g) and quercetin concentration (mg/100 g) of fresh onion leaves were 36.53 ± 2.53 and 5.71 ± 0.11, respectively, and were significantly ($p < 0.05$) higher than those of Welsh onion leaves (25.07 ± 2.02 and 0.18 ± 0.02, respectively). Additionally, total vitamin C (23.36 ± 0.62 mg/100 g) and β-carotene (1529.32 ± 167.77 μg/100 g) concentrations in fresh onion leaves were similar to those rich vegetables.

Conclusions: This is the first study to report fresh onion leaves' sensory properties and volatile compounds. Additionally, fresh onion leaves could be a new food resource, presenting an alternative to Welsh onion leaves and acting as an excellent dietary source of antioxidants, including quercetin, vitamin C, and β-carotene.

Keywords: Fresh onion (*Allium cepa* L.) leaves, Taste components, Volatile compounds, Sensory property, Antioxidants, Quercetin

Background

The genus *Allium* belongs to the Amaryllidaceae family and contains more than 1000 species, including onions (*Allium cepa* L.), garlic (*Allium sativum* L.), chives (*Allium schoenoprasum* L.), leeks (*Allium porrum* L.), and Welsh onion (*Allium fistulosum* L.) (Asemani et al. 2019; Khassanov 2018). Many studies have shown that several *Allium* species contain

*Correspondence: yuasa@people.kobe-u.ac.jp

[1] Graduate School of Human Development and Environment, Kobe University, 3-11 Tsurukabuto, Nada-ku, Kobe, Hyogo 657-8501, Japan
Full list of author information is available at the end of the article

high levels of phenolic compounds, flavonoids, and organosulfur compounds, which possess antioxidative, antimicrobial, anti-inflammatory, hypolipidemic, antidiabetic, cardioprotective, neuroprotective, and anticancer activities (Bastaki et al. 2021; Kurnia et al. 2021; Kothari et al. 2020; Fredotović and Puizina 2019; Putnik et al. 2019). In many countries, *Allium* species are used as spices and food. However, different parts of these species are used in limited areas. For example, onion (*A. cepa* L.) is a popular vegetable consumed worldwide, whereas Welsh onion (*A. fistulosum* L.) is an indispensable ingredient for flavoring many Asian dishes in China, Japan, and Korea.

Onions are dietary sources of quercetin (Nishimuro et al. 2015), dietary fiber, and potassium (MEXT 2020). In Japan, onions are harvested from summer to autumn and spring; for the latter, the onions are harvested from April to May (early-season variety) and before April (very early-season variety) (Yuasa et al. 2020). In Japan, these early-season variety onions are called "fresh onion." However, the leaves of fresh onion (fresh onion leaves) are discarded before shipment to Japan's markets due to their short shelf life. Thus, they are considered food waste in Japan. Fresh onion leaves contain high levels of antioxidants, including total phenolic content (TPC), quercetin, total vitamin C, β-carotene, and chlorophylls (Yuasa et al. 2021; El-Hadidy et al. 2014). Higher levels of carotenoids, TPC, total flavonoids, chlorophylls (El-Hadidy et al. 2014), total vitamin C, and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity (Yuasa et al. 2018) in fresh onion leaves were observed compared with the bulbs (the edible part). Previous studies have suggested that a high intake of total phenolic was a lower risk of diabetes (Tresserra-Rimbau et al. 2016) and was associated with low cardiovascular and digestive disease mortality (Taguchi et al. 2020). Quercetin has anti-obesity (Dong et al. 2014) and antihypertensive effects (Egert et al. 2009). Therefore, the antioxidants in fresh onion leaves may help maintain good health. Conversely, fresh onion leaves taste sour, are less bitter, and have sugar concentrations lower than that of Welsh onion (green leaf, small variety) leaves, which is a vegetable similar to fresh onion leaves (Yuasa et al. 2021). However, reports on the fresh onion leaves' taste, aroma, sensory, and antioxidant properties are very limited. Therefore, to evaluate the fresh onion leaves' usefulness as a new food resource, though a food waste, we characterized their taste characteristics, volatile compounds, sensory properties, and antioxidant activity, compared with those of Welsh onion leaves.

Methods

Sample preparation

Fresh onions (*A. cepa* L.) of the very early-season variety of the Ebisu-dama cultivar were purchased with leaves intact from the Shima-to-kurasu K.K. in Awaji city, Hyogo prefecture, Japan, in February 2019, and the leaves were sampled from the bulbs. Welsh onion (*A. fistulosum* L.) (green leaf, small variety) leaves, with taste similar to fresh onion leaves (Yuasa et al. 2018), were purchased from the Niku-no-nagamoto Co., Ltd. (the local supermarket) in Nagasaki City, Nagasaki prefecture, Japan, in February 2019. All samples were washed using detergent, disinfected using sodium hypochlorite, and then washed extensively in tap water. The samples were dried using a paper towel and then placed in a freezer at − 30 °C for 2 weeks. The frozen samples were freeze-dried and then stored at − 30 °C until further use.

Brix, sugars, and degree of sweetness

One hundred milligrams of the sample was homogenized with 5 mL ultrapure water. The samples were centrifuged at 3000 rpm for ten minutes, and the supernatant was collected for analysis.

Brix value measurements were determined using a Pocket Saccharimeter APAL-1 (As One Corporation., Osaka, Japan). The D-glucose, fructose, and sucrose concentrations were measured using an F-kit (D-Glucose Sucrose Fructose) (Roche Diagnostics K.K., Tokyo, Japan) following the manufacturer's instructions. The Brix value and sugar concentrations were expressed as a percentage and in g per 100 g fresh weight, respectively.

The sweetness intensities of D-glucose and fructose were 0.65-fold and 1.25-fold greater than that of sucrose, respectively. Thus, the "degree of sweetness" was calculated as a measure of the total sweetness using the following formula (Yuasa et al. 2021):

$$\begin{aligned}\text{Degree of sweetness} = &\left(\text{D} - \text{glucose concentration} \times 0.65\right)\\ &+ (\text{fructose concentration} \times 1.25)\\ &+ (\text{sucrose concentration} \times 1.00)\end{aligned}$$

Organic acids

Organic acids were detected using a Prominence Ultra-Fast Liquid Chromatography (UFLC) system (Shimadzu Co., Ltd., Kyoto, Japan), as described previously (Yuasa et al. 2021). A YMC-Triart C18 column (3.0-mm I.D. × 150 mm, 3 μm) (YMC Co., Ltd., Kyoto, Japan) was used for the separation. The sample was homogenized using ultrapure water and centrifuged at 3000 rpm for ten minutes. The supernatant was collected and filtered through a 0.22-μm nylon filter (Starlab Scientific Co.,

Ltd., Shaanxi, China) and analyzed under the following conditions: mobile phase, 20 mmol/L phosphoric acid; injection volume, 6 µL; flow rate, 0.425 mL/min; column oven temperature, 37 °C; and UV/Vis detection wavelength, 220 nm. As presented in Table 1, eight organic acids were detected. The values were expressed in milligrams per 100 g fresh weight.

Table 1 Taste components concentration in leaves of Welsh onion and fresh onion

	Welsh onion leaves	Fresh onion leaves
Brix (%)	7.51 ± 0.31	5.87 ± 0.18*
Sugars (g/100 g)		
D-glucose	0.91 ± 0.04	0.91 ± 0.08
Fructose	1.11 ± 0.07	0.97 ± 0.06*
Sucrose	0.36 ± 0.09	0.23 ± 0.07*
Total	2.38 ± 0.07	2.12 ± 0.15*
Degree of sweetness	2.34 ± 0.07	2.04 ± 0.14*
Organic acids (mg/100 g)		
L-tartaric acid	31.53 ± 0.75	12.96 ± 0.30*
Formic acid	13.89 ± 0.26	13.67 ± 0.44
L-malic acid	738.11 ± 27.22	612.07 ± 14.13*
Lactic acid	15.13 ± 1.79	14.95 ± 1.54
Acetic acid	26.07 ± 5.56	31.39 ± 1.68
Citric acid	45.02 ± 1.49	35.23 ± 1.23*
Succinic acid	7.01 ± 0.16	3.05 ± 0.25*
Fumaric acid	30.47 ± 3.44	6.71 ± 0.30*
Total	907.23 ± 20.79	730.02 ± 17.43*
Free amino acids (mg/100 g)		
L-aspartate	10.10 ± 0.94	5.33 ± 0.72*
L-glutamate	6.12 ± 0.72	7.40 ± 0.89*
L-serine	50.31 ± 2.93	35.15 ± 2.01*
Glycine	0.77 ± 0.04	0.31 ± 0.02*
L-histidine	1.26 ± 0.09	0.93 ± 0.09*
L-arginine	2.44 ± 0.09	0.97 ± 0.08*
L-threonine	4.45 ± 1.13	3.72 ± 0.36
L-alanine	9.21 ± 1.31	4.64 ± 0.75*
L-proline	4.32 ± 0.37	2.53 ± 0.25*
L-tyrosine	2.09 ± 0.36	0.81 ± 0.19*
L-valine	4.92 ± 0.97	2.35 ± 0.38*
L-methionine	5.21 ± 0.28	4.23 ± 0.30*
L-cystine	0.95 ± 0.09	0.80 ± 0.07*
L-isoleucine	11.11 ± 0.66	9.21 ± 0.44*
L-leucine	5.52 ± 0.99	3.38 ± 0.67*
L-phenylalanine	9.19 ± 0.45	8.77 ± 0.42
L-lysine	3.36 ± 0.64	3.20 ± 0.33
Total	131.34 ± 10.22	93.72 ± 7.17*
5′-Guanylate (mg/100 g)	0.35 ± 0.05	0.57 ± 0.05*

Values are the mean ± SD ($n = 5$) * $p < 0.05$ (Welch's t test), compared between Welsh onion leaves and flesh onion leaves in each value

Free amino acids

Free amino acids were detected using a Prominence UFLC system (Shimadzu Co., Ltd., Kyoto, Japan), as previously described (Yuasa et al. 2021). An Inertsil ODS-3 column (4.6 mm i.d. × 150 mm, 3 µm particle size) (GL Sciences Inc., Tokyo, Japan) and an Inertsil ODS cartridge guard column (4.0 mm i.d. × 10 mm, 3 µm particle size) (GL Sciences Inc., Tokyo, Japan) were used for the separation. The sample was homogenized using 0.1 mol/L HCl and centrifuged at 15000 rpm for 10 minutes at 4 °C for supernatant collection. Then, the free amino acids in the supernatant were derivatized to phenylthiocarbamyl (PTC) amino acids using phenyl isothiocyanate. The derivatized sample was dried and dissolved in 0.5 mL mobile phase A and filtered through a 0.22-µm nylon filter (Starlab Scientific Co., Ltd., Shaanxi, China) for analysis. The analysis conditions were as follows: mobile phase A, 60 mmol/L acetate buffer solution (pH 6.6)/acetonitrile (94:6, by vol.); mobile phase B, 60 mmol/L acetate buffer solution (pH 6.6)/acetonitrile (40:60, by vol.); gradient, 0%–55% mobile phase B (0–20 min), 55%–100% mobile phase B (20–25 min), 100% mobile phase B (25–45 min); injection volume, 6 µL; flow rate, 0.6 mL/min; column oven temperature, 40 °C; and UV/Vis detection wavelength, 250 nm. As presented in Table 1, 17 free amino acids were detected. The concentrations were expressed in milligrams per 100 g of fresh weight.

5′-Guanylate

5′-Guanylate was detected using a Prominence UFLC system (Shimadzu Co., Ltd., Kyoto, Japan), as described previously (Yuasa et al. 2021). A Shim-pack WAX-1 column (4.0 mm i.d. × 5.0 mm, 3 µm particle size) (Shimadzu Co., Ltd., Kyoto, Japan) was used for separation. The sample was homogenized using ultrapure water and centrifuged at 3000 rpm for 10 minutes. The supernatant was filtered through a 0.22-µm nylon filter (Starlab Scientific Co., Ltd., Shaanxi, China) and analyzed under the following conditions: mobile phase, 50 mmol/L phosphate buffer (pH 3.1); injection volume, 10 µL; flow rate, 1.0 mL/min; column oven temperature, 40 °C; and UV/Vis detection wavelength, 260 nm. The values were expressed in milligrams per 100 g fresh weight.

Taste-active value (TAV)

The TAV indicates the impact of the individual taste-active compounds in foods and prepared food dishes (Gao et al. 2021; Duan et al. 2020; Keutgen and Pawelzik 2007). The TAV was calculated as the ratio of the actual concentration of taste components and thresholds for the given sugars, organic acids, free amino acids, and 5′-guanylate. The taste thresholds were referenced

from Duan et al. (2020), Kong et al. (2017), Keutgen and Pawelzik (2007), and Kato et al. (1989). If a taste component has a TAV value > 1, it is considered to influence taste.

Taste responses

The taste responses were detected using the Taste Sensing System TS-5000Z (Intelligent Sensor Technology, Inc., Kanagawa, Japan) with artificial lipid-based membrane sensors [CA0 (sourness), C00 (acidic bitterness), AE1 (astringency), AAE (umami), and CT0 (saltiness)] and ceramic reference electrodes, as described previously (Yuasa et al. 2021). The sample was homogenized using ultrapure water. Then, the sample was filtered through a filter paper (No. 5C, Advantech Co., Ltd., Osaka, Japan), and the filtrate was analyzed. The initial taste responses (sourness, acidic bitterness-A, astringency-A, umami, and saltiness) and the aftertaste responses (acidic bitterness-B, astringency-B, and richness) were detected. The values of fresh onion leaves were expressed as fold change relative to those of Welsh onion leaves (average value = 0).

Volatile compounds

The volatile compounds were measured using a headspace solid-phase microextraction (SPME)–gas chromatography (GC)–mass spectrometry (MS)–olfactometry (O) system (GC-MSD; 7890A GC and 5975C inert MSD) (Agilent Technologies, Inc., California, USA) with a DB-WAX column (0.25 mm I.D. × 60 m, 0.25 μm). The Foundation for Promotion of Material Science and Technology of Japan (Tokyo, Japan) performed the SPME-GC-MS-O analysis. The volatile compounds were identified through comparison with mass spectrometry libraries (W9N11.L). A trained expert researcher conducted olfactometry separately on each volatile compound. The values were expressed as the relative percentage of each compound peak area to the total peak area.

Sensory evaluation

Generally, Welsh onions are eaten after cooking; thus, we boiled both samples before sensory evaluation. Leaves of Welsh and fresh onions were placed in boiling water for 90 s, cooled in cold water, and squeezed to remove excess water. For sensory evaluation, pieces of 3 cm in length were prepared. The samples were evaluated using Scheffe's paired comparison method by 36 untrained, non-expert Japanese female students in their 20 s, who were undergoing a registered dietitian course. The intensities (texture, sweetness, pungency, and aroma), preferences (color, texture, aftertaste, and taste), and overall judgement were examined on a seven-point scale from − 3 to + 3. The fresh onion leaves' values were expressed as fold change relative to those of Welsh onion leaves (average value = 0).

Colors

The colors of the boiled samples were measured using a color difference meter ZE2000 (Nippon Denshoku Industries Co., Ltd., Tokyo, Japan). The green and white parts of the samples were measured separately. The color scale was used to measure the L^* (dark to light), a^* (green to red), and b^* (blue to yellow) parameters.

The oxygen radical absorbance capacity (ORAC) value

The ORAC value was measured using the OxiSelect ORAC Activity Assay Kit (Cell Biolabs, Inc., CA, USA). The hydrophilic and lipophilic fractions were prepared using phosphate-buffered saline (PBS; pH 7.4) and acetone, as previously described (Yuasa et al. 2021). The ORAC value was measured for the hydrophilic (hydrophilic (H)-ORAC value) and lipophilic fractions (lipophilic (L)-ORAC value). The values were expressed as μmol 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalents (TE) per 100 g fresh weight.

1-Diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity

The DPPH-free radical-scavenging activity was evaluated using a Prominence UFLC system (Shimadzu Co., Ltd., Kyoto, Japan), as previously described (Yuasa et al. 2021). A TSKgel Octyl-80Ts column (4.6 mm i.d. × 150 mm, 5 μm particle size) (Tosoh Corporation, Tokyo, Japan) was used for the separation. The sample was homogenized in 100 mmol/L Tris–HCl buffer (pH7.4). The homogenized sample was centrifuged at 3000 rpm for 10 minutes, and the supernatant was collected. The supernatant was reacted with 500 μmol/L DPPH solution. The sample was filtered using a 0.22-μm nylon filter (Starlab Scientific Co., Ltd., Shaanxi, China) and analyzed under the following conditions: mobile phase, 70% (v/v) methanol; injection volume, 10 μL; flow rate, 1.0 mL/min; column oven temperature, 40 °C; and UV/Vis detection wavelength, 517 nm (0.064 AUFS). The antioxidant activity of the sample was evaluated from the DPPH concentration. The values were expressed as Trolox equivalent (TE) per 100 g fresh weight.

Total phenolic content (TPC)

As previously described, TPC was determined using the Folin–Ciocalteu method (Yuasa et al. 2021). The extract for analysis was prepared using 80% (v/v) methanol containing 0.5% (v/v) HCl. The values were expressed as mg gallic acid equivalents (GAE) per 100 g fresh weight.

Quercetin and kaempferol

Quercetin and kaempferol were detected using a Prominence UFLC system (Shimadzu Co., Ltd., Kyoto, Japan), as previously described (Aoyama and Yamamoto, 2006). An Inertsil ODS-3 column (4.6 mm i.d. × 150 mm, 5 µm particle size) (GL Sciences Inc., Tokyo, Japan) was used for separation. The extract for analysis was prepared using 1% (w/v) butylhydroxytoluene (BHT) in 80% (v/v) ethanol. Subsequently, the extract, 6 mol/L HCl, and 1% (w/v) BHT in 80% (v/v) ethanol were mixed and then reacted at 90 °C for 120 min. Then, the sample was filtered using a 0.22-µm PTFE filter (Starlab Scientific Co., Ltd., Shaanxi, China). The analysis conditions were as follows: mobile phase, acetonitrile: 0.025 mol/L KH_2PO_4 (pH2.4) (30:70 by vol.); injection volume, 10 µL; flow rate, 1.3 mL/min; column oven temperature, 30 °C; and UV/Vis detection wavelength, 370 nm. The values were expressed as milligrams per 100 g fresh weight.

Total vitamin C

Total vitamin C was detected using the Prominence UFLC system (Shimadzu Co., Ltd., Kyoto, Japan), as previously described (Yuasa et al. 2021). An Inertsil SIL-100A column (4.6 mm i.d. × 250 mm, 5 µm particle size) (GL Sciences Inc., Tokyo, Japan) was used for separation. One hundred milligrams of the sample was homogenized in 20 mL 5% (w/v) metaphosphoric acid, made up to 30 mL using 5% (w/v) metaphosphoric acid. The homogenized sample was centrifuged at 3000 rpm for 10 minutes, and the supernatant was collected. Subsequently, vitamin C in the supernatant was reacted using 2,4-dinitrophenylhydrazine to form the derivative bis-2,4-dinitrophenylhydrazone and then transferred to the ethyl acetate. The ethyl acetate layer was dehydrated using anhydrous sodium sulfate and filtered using a 0.22-µm nylon filter (Starlab Scientific Co., Ltd., Shaanxi, China). The analysis conditions were as follows: mobile phase, ethyl acetate/hexane/acetate (50: 40: 10 by vol.); injection volume, 10 µL; flow rate, 1.5 mL/min; column oven temperature, 40 °C; and UV/Vis monitoring wavelength, 495 nm. This method measures the total amount of ascorbic and dehydroascorbic acids. The values were expressed as milligrams per 100 g fresh weight.

Carotenoids and chlorophylls

The carotenoids and chlorophylls were detected using a Prominence UFLC system (Shimadzu Co., Ltd., Kyoto, Japan), as previously described (Yuasa et al. 2021). An Inertsil ODS-3 column (4.6 mm i.d. × 150 mm, 5 µm particle size) was used for separation. The sample was extracted using acetone and filtered through a 0.22-µm nylon filter (Starlab Scientific Co., Ltd., Shaanxi, China). The following analysis conditions were used: mobile phase A, 90% (v/v) acetonitrile; mobile phase B, ethyl acetate; 0–50% mobile phase B (0–7.8 min), 50% mobile phase B (7.8–13.8 min), 0% mobile phase B (13.8–22.0 min); injection volume, 10 µL; flow rate, 1.5 mL/min; column oven temperature, 40 °C; and UV/Vis detection wavelength, 450 nm. Carotenoids and chlorophylls were detected, as presented in Table 5. The values were expressed as µg (carotenoids) or mg (chlorophylls) per 100 g fresh weight.

Statistical analysis

The values are presented as the mean or the mean ± standard deviation (SD). Statistical analysis was conducted using Excel 2019 (Microsoft Japan Co., Ltd., Tokyo, Japan) and EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 4.0.3) (Kanda 2013). The statistical significance of differences was compared using the Welch's t test for experimental data, Scheffe's method of paired comparison for sensory evaluation, and Spearman's rank correlation coefficient for the correlation between taste responses and components. A p value of < 0.05 was considered significant.

Results

Taste characteristics of fresh onion leaves

The concentrations of taste components in the Welsh and fresh onion leaves are presented in Table 1. Lower Brix levels, lower fructose, sucrose, and total sugar concentrations, and a lower degree of sweetness were found in fresh onion leaves ($p < 0.05$) than in Welsh onion leaves. The concentrations of L-tartaric, L-malic, citric, succinic, and fumaric acids were lower in fresh onion leaves than in Welsh onion leaves ($p < 0.05$). In fresh onion leaves, the concentrations of L-aspartate, L-serine, glycine, L-histidine, L-arginine, L-alanine, L-proline, L-tyrosine, L-valine, L-methionine, L-cystine, L-isoleucine, L-leucine, and total free amino acids were lower than in Welsh onion leaves, whereas L-glutamate was higher ($p < 0.05$). The 5'-guanylate concentration in fresh onion leaves was higher than that in Welsh onion leaves ($p < 0.05$). The taste responses to the Welsh and fresh onion leaves are shown in Fig. 1. A lower level of initial taste astringency-A was observed in fresh onion leaves than in Welsh onion leaves ($p < 0.05$). The value of astringency-A correlated positively with the concentrations of L-aspartate, L-arginine, L-threonine, L-alanine, L-valine, L-methionine, L-isoleucine, L-leucine, and total free amino acids ($r = 0.661–0.733$, $p < 0.05$).

The TAVs of taste components in the Welsh and fresh onion leaves are presented in Table 2. The taste components in both samples, with TAVs higher than 1, were

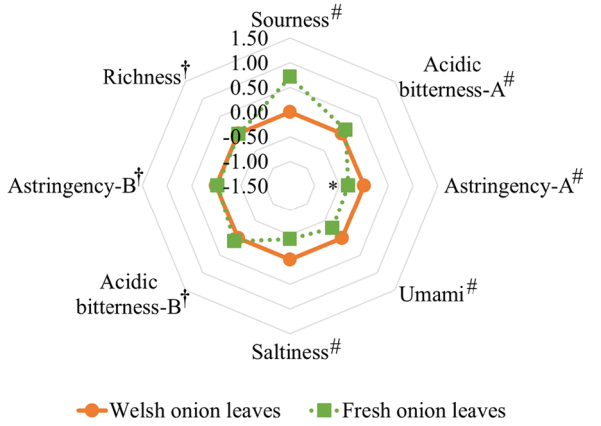

Fig. 1 Taste responses in leaves of Welsh onion and fresh onion. #Initial taste, †Aftertaste. Values are expressed as fold change relative to Welsh onion leaves each taste (Welsh onion leaves = 0). Values are the mean ($n = 5$). * $p < 0.05$ (Welch's t test), compared between Welsh onion leaves and flesh onion leaves in each taste

fructose, L-tartaric acid, L-malic acid, and acetic acid. Additionally, the TAV of citric acid in Welsh onion leaves was greater than 1.

Volatile compounds in fresh onion leaves

The percentages of the peak areas of volatile compounds and the GC–MS total ion chromatogram in the Welsh and fresh onion leaves are presented in Table 3 and Fig. 2, respectively. The total ion chromatograms of the volatile aroma compounds were similar in both samples. Twenty-seven and 24 volatile compounds were observed in the Welsh and fresh onion leaves, respectively. In fresh onion leaves, peaks isovaleraldehyde (No. 1) and unknown (No. 2) were less than half of those in Welsh onion leaves, and peak dipropyl disulfide (No. 19) was more than five times that of Welsh onion leaves. In the olfactometry experiments, the aromas of diacetyl (No. 4), dipropyl disulfide (No. 19), and 3,4-dimethylisothiazole (No. 20) were sensed.

Sensory properties and colors of boiled fresh onion leaves

The results of the sensory evaluation of boiled Welsh and fresh onion leaves are shown in Fig. 3. The color score of fresh onion leaves was lower than that of Welsh onion leaves ($p < 0.05$). No differences between the samples were found for the other sensory properties.

The results of the Welsh and fresh onion leaves' color after boiling treatment are shown in Table 4. In the green part, the a^* and L^* values were higher and the b^* value was lower in fresh onion leaves than in Welsh onion leaves ($p < 0.05$). In the white part, the L^*, a^*, and b^* values of fresh onion leaves were lower than in Welsh onion leaves ($p < 0.05$).

Table 2 Taste-active value (TAV) of taste components in leaves of Welsh onion and fresh onion

	Taste threshold† (mg/100 g)	TAV	
		Welsh onion leaves	Fresh onion leaves
Sugars#			
D-glucose	1.62	0.56	0.56
Fructose	0.94	1.18	1.04
Sucrose	0.82	0.43	0.28
Organic acids			
L-tartaric acid	1.5	21.02	8.64
Formic acid	200.0	0.07	0.07
L-malic acid	49.6	14.88	12.34
Lactic acid	126.0	0.12	0.12
Acetic acid	10.6	2.46	2.96
Citric acid	45.0	1.00	0.78
Succinic acid	10.6	0.66	0.29
Free amino acids			
L-aspartate	100.0	0.10	0.05
L-glutamate	30.0	0.20	0.25
L-serine	150.0	0.34	0.23
Glycine	130.0	0.01	0.00
L-histidine	20.0	0.06	0.05
L-arginine	50.0	0.05	0.02
L-threonine	260.0	0.02	0.01
L-alanine	60.0	0.15	0.08
L-proline	300.0	0.01	0.01
L-tyrosine	884.0	0.00	0.00
L-valine	40.0	0.12	0.06
L-methionine	30.0	0.17	0.14
L-isoleucine	90.0	0.12	0.10
L-leucine	190.0	0.03	0.02
L-phenylalanine	90.0	0.10	0.10
L-lysine	50.0	0.07	0.06
5′-Guanylate	12.5	0.03	0.05

Unit of sugars (D-glucose, fructose, and sucrose) shown as g/100 g

† Taste thresholds of taste components were referred from Duan et al. (2020), Kong et al. (2017), Keutgen and Pawelzik (2007), and Kato et al. (1989). Values are the mean ($n = 5$)

Antioxidant activity and antioxidant concentrations in fresh onion leaves

The antioxidant activity and antioxidant concentrations in the Welsh and fresh onion leaves are shown in Table 5. The H-ORAC, L-ORAC, and total (T)-ORAC values and DPPH-free radical-scavenging activity differed insignificantly between the Welsh and fresh onion leaves. A substantial component of the total ORAC value was the H-ORAC value (water-soluble fraction).

In fresh onion leaves, the total phenolic and quercetin concentrations were higher than in Welsh onion

Table 3 Volatile compounds and odor characteristics in leaves of Welsh onion and fresh onion

No.	Compounds	Retention time (min)	Area (%)		Odor characteristics
			Welsh onion leaves	Fresh onion leaves	
1	Isovaleraldehyde	4.311	3.36	1.01	
2	Unknown	4.722	2.64	1.22	
3	Ethanol	4.786	7.92	6.56	
4	Diacetyl	5.637	13.57[#]	9.24[#]	Welsh onion-like, unpleasant odor
5	n-Pentanal	5.664			
6	2,2,4,6,6-Pentamethylheptane	6.144	6.80	7.08	
7	Unknown	6.559	10.77	11.62	
8	Unknown	7.232	7.40	10.29	
9	Unknown	7.563	7.74	11.77	
10	n-Hexanal	8.691	3.45	6.22	
11	2-Methyl-2-Butenal	9.007	3.21	2.39	
12	3-Penten-2-one	10.126	1.41	LOD	
13	Unknown	11.085	1.59	1.67	
14	1-Penten-3-ol	11.550	2.25	1.27	
15	Dodecane	12.839	1.18	LOD	
16	2-Hexenal	13.310	0.27	0.53	
17	Acetoin	15.662	6.87	11.23	
18	1,1,3-Trimethyl-2-cyclohexanone	16.697	0.46	0.55	
19	Dipropyl disulfide	18.765	0.52	2.65	Welsh onion-like, fishy smell
20	3,4-Dimethylisothiazole	19.917	0.45	0.63	Soil-like
21	3-Methoxy-3-methylbutanol	20.749	5.52	LOD	
22	Acetic acid	21.220	6.56	7.88	
23	2-Decanone	22.520	LOD	0.75	
24	Benzaldehyde	23.235	2.45[†]	1.64[†]	
25	3,5-Octadiene-2-one	23.267			
26	Propanoic acid	23.921	1.21	1.25	
27	2,3-Butanediol	24.064	2.05	2.54	
28	2-Methylbutanoic acid	27.767	0.36	LOD	
	Number of compounds		27	24	

LOD, limit of detection

[#] Sum of area (%) of diacetyl and n-pentanal

[†] Sum of area (%) of benzaldehyde and 3,5-octadiene-2-one

leaves ($p < 0.05$), and the kaempferol, β-carotene, β-cryptoxanthin, chlorophyll a, and chlorophyll b concentrations were lower ($p < 0.05$). The total vitamin C concentration was indifferent between the samples, and α-carotene was undetected.

Discussion

In this study, to evaluate the usefulness of fresh onion leaves as a new food resource, though a waste food, we characterized their taste characteristics, volatile compounds, sensory properties, and antioxidant activity, compared with those of Welsh onion leaves.

We showed that the values of Brix, fructose, sucrose, total sugars, degree of sweetness, and initial taste astringency-A were lower in fresh onion leaves than in Welsh onion leaves. These results suggest that the sweetness and initial taste astringency were lower in fresh onion leaves than in Welsh onion leaves. The lower levels of initial taste astringency in fresh onion leaves may result from the different concentrations of the various organic acids, free amino acids, and 5'-guanylate between the samples. In previous reports, the values of the initial taste astringency correlated positively with the free amino acid concentrations in eggplants (Soga et al. 2009). In this study, the value of astringency-A correlated positively

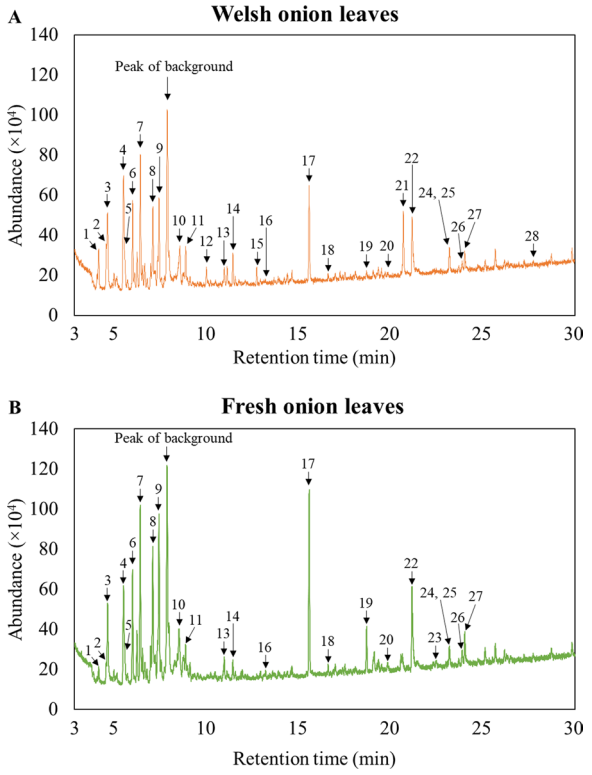

Fig. 2 GC–MS total ion chromatogram of volatile compounds in leaves of Welsh onion (**A**) and fresh onion (**B**). All the peaks were numbered on the basis of the order number of each compound in Table 3

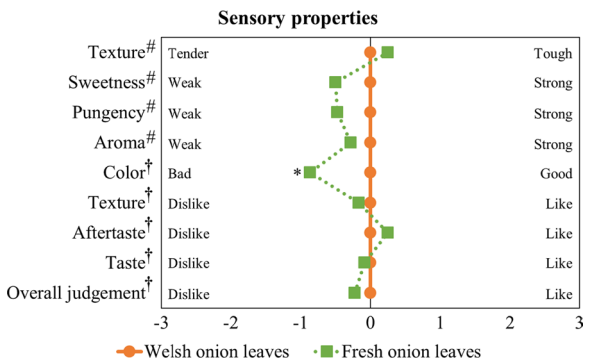

Fig. 3 Comparison of sensory properties in leaves of Welsh onion and fresh onion. #Intensity, †preference. Values are expressed as fold change relative to Welsh onion leaves each evaluation (Welsh onion leaves = 0). Values are the means ($n = 36$). * $p < 0.05$ (Scheffe's method of paired comparison), the scores for each evaluation for fresh onion leaves were evaluated when the Welsh onion leaves were considered to be 0

with some amino acid concentrations. Our study's results and previous studies suggest that the higher level of initial taste astringency in Welsh onion leaves was induced by higher levels in the concentrations of free amino and organic acids compared with fresh onion leaves. Additionally, the astringency sensor AE1 responded to phenolic compounds, such as tannic acid, gallic acid, caffeic acid, and epigallocatechin gallate (Kobayashi et al. 2010). However, in this study, TPC was lower in Welsh onion leaves than in fresh onion leaves. Thus, the phenolic compounds may not affect the astringency of the samples.

For humans to discriminate differences in taste, the differences in the taste response values should be more than one. The difference between the Welsh and fresh onion leaves did not exceed one for any taste responses. Furthermore, our study's results revealed that the taste responses of fresh onion leaves were closer to those of Welsh onion than those of Chinese chives (Yuasa et al. 2018). Therefore, the bitterness, astringency, umami, saltiness, and richness of fresh onion leaves were similar to those of Welsh onion leaves. We suggested this observation in our previous study (Yuasa et al. 2021) and confirmed it in this study.

In this study, the major taste components in the Welsh and fresh onion leaves, with TAVs higher than 1, were fructose, L-tartaric acid, L-malic acid, and acetic acid. The TAVs of sugars and organic acids in strawberries (Keutgen and Pawelzik 2007) and many fruit beverages (Dias et al. 2014) were more than one. In commercial white tea extracts, the TAVs of free amino acids were lower than 0.04 (Yang et al. 2018). Our results agree with previous results, suggesting that sugars and organic acids, rather than free amino acids, are the main taste-active compounds in plant-based foods, such as vegetables, fruits, and tea. In contrast, in some vegetables with lower L-glutamate concentrations than the taste threshold of L-glutamate, the hedonic values and the L-glutamate concentration were correlated (Baryłko-Pikielna and Kostyra 2007). In boiled potato, the equivalent umami scores, calculated from L-glutamate, L-aspartate, 5'-guanylate, and 5'-adenylate concentrations, correlated with sensory evaluation scores, such as flavor intensity, acceptability, sweet flavor, and creamy flavor (Morris et al. 2007). Thus, although the concentrations of taste components of simple substances were lower, taste interactions were observed in the complex food matrix. Therefore, the interaction of taste components in the Welsh and fresh onion leaves should be investigated in the future.

In this study, the total ion chromatograms of the volatile aroma compounds were similar in the Welsh and fresh onion leaves. Thus, the profiles of volatile aroma compounds in the Welsh and fresh onion leaves were similar. The main volatile aroma compounds were diacetyl (Welsh onion-like, unpleasant odor), dipropyl disulfide (Welsh onion-like, fishy smell), and

Table 4 Colors of leaves of Welsh onion and fresh onion after boiling treatment

	Green part		White part	
	Welsh onion leaves	**Fresh onion leaves**	**Welsh onion leaves**	**Fresh onion leaves**
L^*	23.91 ± 0.04	24.34 ± 0.02*	69.09 ± 0.02	54.84 ± 0.03*
a^*	-17.36 ± 0.07	− 10.13 ± 0.03*	− 7.37 ± 0.03	− 7.96 ± 0.03*
b^*	20.69 ± 0.11	16.04 ± 0.07*	22.68 ± 0.01	18.84 ± 0.01*

Values are the mean ± SD ($n = 15$). * $p < 0.05$ (Welch's *t* test), compared between Welsh onion and flesh onion leaves in each value

Table 5 Antioxidant activity and antioxidants concentration in leaves of Welsh onion and fresh onion

	Welsh onion leaves	**Fresh onion leaves**
ORAC values (μmol TE/100 g)		
Hydrophilic (H)—ORAC	844.36 ± 112.72	762.13 ± 103.82
Lipophilic (L)—ORAC	43.63 ± 1.36	43.65 ± 8.95
Total (T)—ORAC	888.00 ± 112.61	805.78 ± 100.32
DPPH-free radical-scavenging activity (μmol TE/100 g)	382.98 ± 26.08	406.70 ± 63.64
Total phenolic content (mg GAE/100 g)	25.07 ± 2.02	36.53 ± 2.53*
Quercetin (mg/100 g)	0.18 ± 0.02	5.71 ± 0.11*
Kaempferol (mg/100 g)	2.14 ± 0.03	0.86 ± 0.02*
Total vitamin C (mg/100 g)	24.10 ± 0.45	23.36 ± 0.62
Carotenoids (μg/100 g)		
α-carotene	LOD	LOD
β-carotene	2420.56 ± 113.51	1529.32 ± 167.77*
β-cryptoxanthin	10.11 ± 0.80	7.44 ± 1.39*
Chlorophylls (mg/100 g)		
Chlorophyll a	10.35 ± 0.16	5.96 ± 0.17*
Chlorophyll b	27.27 ± 0.56	16.82 ± 0.50*

LOD, limit of detection. TE, Trolox equivalents. GAE, gallic acid equivalents. Values are the mean ± SD ($n = 5$). * $p < 0.05$ (Welch's *t* test), compared between Welsh onion leaves and flesh onion leaves in each value

3,4-dimethylisothiazole (soil-like). Additionally, the difference in volatile aromas may result from the different dipropyl disulfide concentrations. Dipropyl disulfide was previously detected in Welsh onions (Nielsen and Poll 2004; Kuo and Ho 1992). The dipropyl disulfide concentration was higher than the other aroma compounds in onions (*Allium cepa* L.) (Cecchi et al. 2020; Järvenpää et al. 1998). These reports indicate that the main aroma compound in fresh and Welsh onion leaves was dipropyl disulfide. This compound was also detected in both fresh and Welsh onion leaves in this study. In contrast, diacetyl and 3,4-dimethylisothiazole have not been previously detected in Welsh onions, onions, and fresh onion leaves; thus, our results are the first indication of this observation.

In the sensory evaluation, we showed that the color score of fresh onion leaves was lower than that of Welsh onion leaves. However, no differences between the samples were found for the other sensory properties. Overall, the taste, aroma, and textural characteristics of boiled fresh and Welsh onion leaves were indifferent. Thus, the sensory properties of fresh onion leaves were similar to those of Welsh onion leaves. The taste and aroma characteristics of these samples agreed with our other study's results, including taste response and volatile compound profile. In contrast, the L^*, a^*, and b^* values differed significantly between the boiled fresh and Welsh onion leaves. In the boiled fresh onion leaves, the green part appeared dark green in color and the white part appeared darker compared with Welsh onion leaves. Therefore, the differences in color appearance may have affected the sensory evaluation of color.

The ORAC values and DPPH-free radical-scavenging activity differed insignificantly between the Welsh and fresh onion leaves. Therefore, the antioxidant activity of the fresh and Welsh onion leaves was approximately similar. These observations were proposed in our previous study (Yuasa et al. 2021) and confirmed by the present results. In this study, the total phenolic and quercetin concentrations were higher, and the kaempferol

concentration was lower in fresh onion leaves than in Welsh onion leaves. The total phenolic and total quercetin concentrations in fresh onion leaves were higher than in Welsh onion (Yuasa et al. 2021). In onions, the quercetin concentration was higher than those in green and white Welsh onions, and the kaempferol concentration was lower (Aoyama and Yamamoto, 2007). The results of both previous studies and the present study showed that the composition of flavonoids was different in fresh onion leaves and Welsh onion leaves: The main flavonoids were quercetin in the former and kaempferol in the latter.

In this study, TPC in fresh onion leaves was higher than in Welsh onion leaves. However, the H-ORAC value, T-ORAC value, and DPPH-free radical-scavenging activity were indifferent between the samples. The antioxidant activity of kaempferol was stronger than that of quercetin in the DPPH-free radical-scavenging assay (Erkan et al. 2011; Hidalgo et al. 2010). The difference in antioxidant activity between quercetin and kaempferol may affect the ORAC value and DPPH-free radical-scavenging activity.

Our results indicate that fresh onion leaves are an excellent dietary source of antioxidants, such as quercetin, vitamin C, and β-carotene. Quercetin is an antioxidant flavonoid (Boots et al. 2008) found in several vegetables (Nishimuro et al. 2015). Quercetin decreases body fat, exerts anti-obesity effects (Dong et al. 2014), and reduces systolic blood pressure and plasma oxidized low-density lipoprotein concentrations (Egert et al. 2009). In humans, quercetin intake is 3.5–16.2 mg/day (Dabeek and Marra 2019; Nishimuro et al. 2015). The average quercetin concentration in fresh onion leaves was 5.71 mg/100 g, representing an excellent dietary source of quercetin. In contrast, β-carotene-rich vegetables, such as tomato, broccoli, *mizuna*, green bok choy, and red leaf lettuce, contain 540–2,000 μg/100 g β-carotene, and vitamin C-rich vegetables, such as tomato, green asparagus, Chinese chive, red leaf lettuce, and green bok choy, contain 15–24 mg/100 g total vitamin C (MEXT, 2020). The fresh onion leaves contain almost the same values of these antioxidants; therefore, fresh onion leaves may be an excellent dietary source of these antioxidants. In future studies, the development of cooking and processing methods or the evaluation of functions in vivo in fresh onion leaves should be conducted.

In a previous study, we reported that total ORAC values, β-carotene, chlorophyll a, and chlorophyll b in fresh onion leaves for the cultivars Super-up and Kazusa No. 13 were 1241.14 and 986.37 μmol Trolox equivalent/100 g, 3903.69 and 3549.83 μg/100 g, 11.92 and 10.83 mg /100 g, and 31.36 and 25.82 mg/100 g, respectively (Yuasa et al. 2021). Notably, the values for the Ebisu-dama cultivar in the present study were lower than those in our previous study. In contrast, the concentrations of total flavonoids, β-carotene, and chlorophylls in fresh onion leaves differed among cultivars (Yuasa et al. 2021; El-Hadidy et al. 2014). It is suggested that the cultivation environment, including the fertilizer regimen, exposure to light, and temperature, affects the antioxidant activity of onions (Ren et al. 2017). Therefore, the differences in antioxidant activity and concentrations between the present and previous studies may be attributable to the differences in the cultivar and the cultivation environments.

In this study, the chemical composition of fresh onion leaves was examined only raw, and the sensory evaluation of samples was conducted only after boiling. However, the chemical composition of fresh onion leaves may be changed by cooking processes. Thus, the chemical composition of the boiled samples and the sensory properties of samples in raw should be investigated in future studies.

Conclusions

In this study, we report the fresh onion leaves' taste characteristics, volatile compounds, sensory properties, and antioxidant activity. We found that some taste components and dipropyl disulfide concentrations in fresh onion leaves differed from those in Welsh onion leaves. However, the sensory properties for each sample were almost similar, except for the color difference. Additionally, the major taste components in fresh onion leaves were fructose, L-tartaric acid, L-malic acid, and acetic acid. The antioxidant activities of the fresh and Welsh onion leaves were indifferent. However, the total phenolic and quercetin concentrations in fresh onion leaves were higher than those in Welsh onion leaves. Additionally, the fresh onion leaves were rich in quercetin, total vitamin C, and β-carotene. This is the first study to report on fresh onion leaves' sensory properties, major taste components, and volatile compounds. Additionally, fresh onion leaves could be a new food resource, presenting an alternative to Welsh onion leaves and acting as an excellent dietary source of antioxidants.

Abbreviations

TAV: Taste-active value; TPC: Total phenolic content; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; ORAC: Oxygen radical absorbance capacity; PBS: Phosphate-buffered saline; Trolox: 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE: Trolox equivalents; GAE: Gallic acid equivalents; PTC: Phenylthiocarbamyl; BHT: Butylhydroxytoluene; UFLC: Ultra-fast liquid chromatography; SPME: Solid-phase microextraction; GC: Gas chromatography; MS: Mass spectrometry; O: Olfactometry; SD: Standard deviation; LOD: Limit of detection.

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Author contributions
MY, MaU, TM, and MT conceived and designed the study. MY, MaU, KK, and MM performed the experiments and sensory evaluation. MY and MaU analyzed the data. MoU, TM, and MT assisted with the experiments and sensory evaluation and analyzed the data. MY wrote the first draft of the manuscript and performed review. All authors read and approved the final manuscript.

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Author details
[1]Graduate School of Human Development and Environment, Kobe University, 3-11 Tsurukabuto, Nada-ku, Kobe, Hyogo 657-8501, Japan. [2]Department of Nutritional Science, Faculty of Nursing and Nutrition, University of Nagasaki, 1-1-1 Manabino, Nagayo-cho, Nishi-Sonogi-gun, Nagasaki 851-2195, Japan. [3]Graduate School of Human Health Science, University of Nagasaki, 1-1-1 Manabino, Nagayo-cho, Nishi-Sonogi-gun, Nagasaki 851-2195, Japan. [4]Department of Health and Nutrition, Faculty of Health Management, Nagasaki International University, 2825-7 Huis Ten Bosch, Sasebo City, Nagasaki 859-3298, Japan. [5]Graduate School of Humanities and Social Sciences, Hiroshima University, 1-1-1 Kagamiyama, Higashi-Hiroshima City, Hiroshima 739-8524, Japan.

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