Journal of Agricultural Science; Vol. 12, No. 2; 2020
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Capsicum Cultivated Under Adverse Conditions Produces High Concentrations of Antioxidants and Capsaicinoids

Katsuko Kajiya[1], Hiroki Yamanouchi[2], Yurika Tanaka[1], Hiroka Hayashi[1] & Yuji Minami[1]

[1] Department of Food Science & Biotechnology, Faculty of Agriculture, Kagoshima University, Kagoshima, Japan

[2] Course of Biological Science & Technology, The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, Japan

Correspondence: Katsuko Kajiya, Department of Food Science & Biotechnology, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan. Tel/Fax: 81-99-285-8631. E-mail: kajiya@chem.agri.kagoshima-u.ac.jp

Received: November 3, 2019 Accepted: December 19, 2019 Online Published: January 15, 2020

doi:10.5539/jas.v12n2p1 URL: https://doi.org/10.5539/jas.v12n2p1

The research is financed by JSPS KAKENHI and a Project of the Bio-Oriented Technology Research Advancement Institution, NARO.

Abstract

Growing crops in sabulous soil is challenging owing to limited oligotrophy and low water retention. Nonetheless, some plants adapt well, imparting favorable properties to the fruit. This study investigated the influence of sandy soil (southern Japan) on red pepper by assessing the levels of pungent components, antioxidant activity, and vascular endothelial function. Leaves and fruits of Habanero orange and Tabasco pepper, the two varieties most suitable for cultivation in sandy soil, were analyzed for size, color, and pungent component composition. Pungent components were detected in the seeds and placenta of fruits but not in leaves or flowers. Antioxidant activity and nitric oxide production in human vascular endothelial cells were evaluated to detect differences in functionality. *Capsicum* peppers cultivated in sandy soil exhibited higher levels of antioxidants than peppers cultivated under nutrient-rich conditions (control) and induced nitric oxide levels in vascular endothelial cells similar to control peppers. Especially *Satsuma*-Habanero orange peppers cultivated in sandy soil exhibited the highest antioxidant activity. The fruits from pepper plants cultivated in sabulous soil could be harvested for a significantly longer period and were slower to spoil than control peppers; therefore, *Satsuma-Capsicum* plants may be commercially viable in oligotrophic areas.

Keywords: antioxidant activity, *Capsicum*, pungent component, red pepper, vascular endothelial function

1. Introduction

The chemical composition and nutritional value of plants vary depending on the cultivation environment (Wakamatsu et al., 2019). Sandy soil in coastal areas lacks nutrients and has low moisture retention, severely limiting the number of crop species that can be grown (Roper et al., 2015). Sandy soil has extremely low clay content and accumulates low amounts of organic matter. Further, although sandy soil possesses good breathability and drainage, it has low water retention and natural fertility (Banedjschafie & Durner, 2015). The temperature of sandy soil tends to rise rapidly. Therefore, it is susceptible to drought, and nutrients from added fertilizer often leak out. Owing to low physical buffering capacity, the typical properties of sandy soil, including earthiness, air and water permeability, water retention, soil temperature, sand scattering, chemicals, nutrient sources, nutrient transfer and absorption, soil pH, pests, and accumulation of organic matter, are strongly influenced by environmental factors (Banedjschafie & Durner, 2015; Roper et al., 2015). Thus, the properties of sandy soil are generally variable and unstable. Consequently, these variations in soil properties could affect the concentration of active components in plants, such as pepper.

Pepper (genus *Capsicum*) belongs to the family Solanaceae. Plants of *Capsicum* spp. are 40-60 cm in height and highly branched. The leaves are mutually alternated with a long petiole and are oval-shaped. After the flowers have bloomed, the plant bears green fruits, having a cavity containing the seeds and placenta (s/p). Depending on

the variety, the fruit may be rounded or shortened, with a wide spectrum of colors. Except for specific sweet pepper species that are generally mild in flavor, many *Capsicum* spp. are favored for their spicy flavor (Monforte-González et al., 2010; Zhang et al., 2016). The primary pungent components of red pepper are capsaicinoids, including capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin (Othman et al., 2011; Reyes-Escogido et al., 2011). In this study, we focused on capsaicin and dihydrocapsaicin as these compounds are mainly responsible for pungency. Plants of the genus *Capsicum* generally contain 60-70% capsaicin, 30-40% dihydrocapsaicin, and other capsaicinoids in trace amounts (Othman et al., 2011; Reyes-Escogido et al., 2011; Zhang et al., 2016). The concentration of pungent components in *Capsicum* spp. varies as a function of light intensity and temperature at which the plant is grown, the age of the fruit, and the relative position of the fruit on the plant (Ng & Reuter, 2015; Othman et al., 2011). The nondestructive identification of pungent component contents is useful for determining the optimum harvest time. However, currently, the fruits are harvested empirically by farmers because determining the optimum harvest time based on capsaicinoids as the pungent component and carotenoids as the primary source of color for the fruit is considered irrelevant. Thus, it is important to understand the changes in capsaicin and dihydrocapsaicin concentrations over time.

It has been reported that, in addition to giving a pungent taste, red peppers have bioregulatory functions, such as antioxidant activity (Sim & Sil, 2008), and contribute to vascular endothelial function improvement (McCarty et al., 2015). These functions may be further enhanced by sandy soil cultivation. Oxidative stress, caused by excessive production of active oxygen within the body, is a risk factor of obesity and lifestyle-related diseases. Therefore, the antioxidant properties of foods are important (Sim & Sil, 2008; Song et al., 2010). Further, red peppers increase nitric oxide (NO) release by vascular endothelial cells (VECs) (Chularojmontri et al., 2010; McCarty et al., 2015; Song et al., 2010). NO released from VECs regulates the contraction and relaxation of blood vessels and prevents thrombus formation due to the attachment of white blood cells and other blood components to the vascular endothelium (Kuroda et al., 2018). However, if VECs are damaged by oxidative stress caused by reactive oxygen species or oxidized low-density lipoproteins, NO production is suppressed thereby increasing the risk of cardiovascular disease. Thus, improving NO production by VECs is critical for protecting the blood vessels. It was reported that capsaicin from red pepper has the potential to modulate metabolism via activation of transient receptor potential cation channel subfamily V member 1 (TRPV1), and TRPV1 activation is associated with increased activation or expression of key proteins such as endothelial NO synthase (Wang et al., 2017).

The distribution of pungent components in pepper varieties cultivated in harsh environments has not been investigated to date. Therefore, we compared the concentrations of pungent components in the pericarp and seeds of peppers cultivated in a harsh environment and those in peppers cultivated under optimal conditions. Further, we investigated the antioxidant activity and the effect of peppers on vascular endothelial function by analyzing changes in NO production in VECs.

2. Methods

2.1 Chemicals and Cells

Acetonitrile and capsaicinoids were purchased from Sigma Aldrich (St. Louis, Mo, USA). Ethanol, hexane, dichloromethane, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Fujifilm Wako Chemical Corporation (Osaka, Japan). 2,3-Diaminonaphthalene (DAN) was purchased from Dojindo Laboratories (Kumamoto, Japan). Normal human VECs derived from human coronary artery and porcine VECs derived from a porcine aortic artery were obtained from Kurabou Industries Ltd. (Osaka, Japan) and Cosmo Bio Co., Ltd. (Tokyo, Japan), respectively. The experiments were performed using both human and porcine VECs, but the figures were prepared using the data from the human-VEC experiments, which included a high number of replicates (n = 8).

2.2 Sample Preparation

In total, seven *Capsicum* varieties (Figure 1A) were used in this study, including *Capsicum chinense* (Habanero orange and Habanero red), *Capsicum annuum* (Indonesian origin and Laris), and *Capsicum frutescens* (Taruna pepper, Okinawan chili pepper, and Tabasco pepper). These *Capsicum* varieties were cultivated in the sandy soil of southern Japan (base material: non-consolidated sedimentary rocks or sea sand; texture: from sandy loam to sand; soil color by Munsell system: 7.5 YR 3/3 to 10 YR 6/6; grain: single-grained structure; pH (H_2O): 6.0±0.4). Red peppers cultivated in sandy soil were named "*Satsuma-Capsicum*", the name of the city where they were cultivated (Minami-Satsuma city, Kagoshima, Japan), to distinguish them from the control peppers. *Satsuma-Capsicum* plants from each of the seven varieties were harvested when the coloration of the mature

fruit changed; color change was used as a criterion because of differences in maturation time among the varieties. For comparison, as control, two traditional tropical-origin pepper varieties, namely Habanero orange and Tabasco pepper (originating from the USA), were provided by Dr. Jun-Ichi Sakagami and Mr. Kenta Komori from the Laboratory of Tropical Crop Science, Faculty of Agriculture, Kagoshima University (Japan). These control peppers were cultivated in nutrient-rich soils at Kagoshima University Experimental Farm. The pericarp and s/p (Figure 1B) were carefully dissected from the peppers, placed on a glass petri dish, and dried in a forced convection oven (DO-60FA; AS ONE, Osaka, Japan) at 60 °C for 13 h. The thoroughly dried materials were crushed using a mortar and pestle.

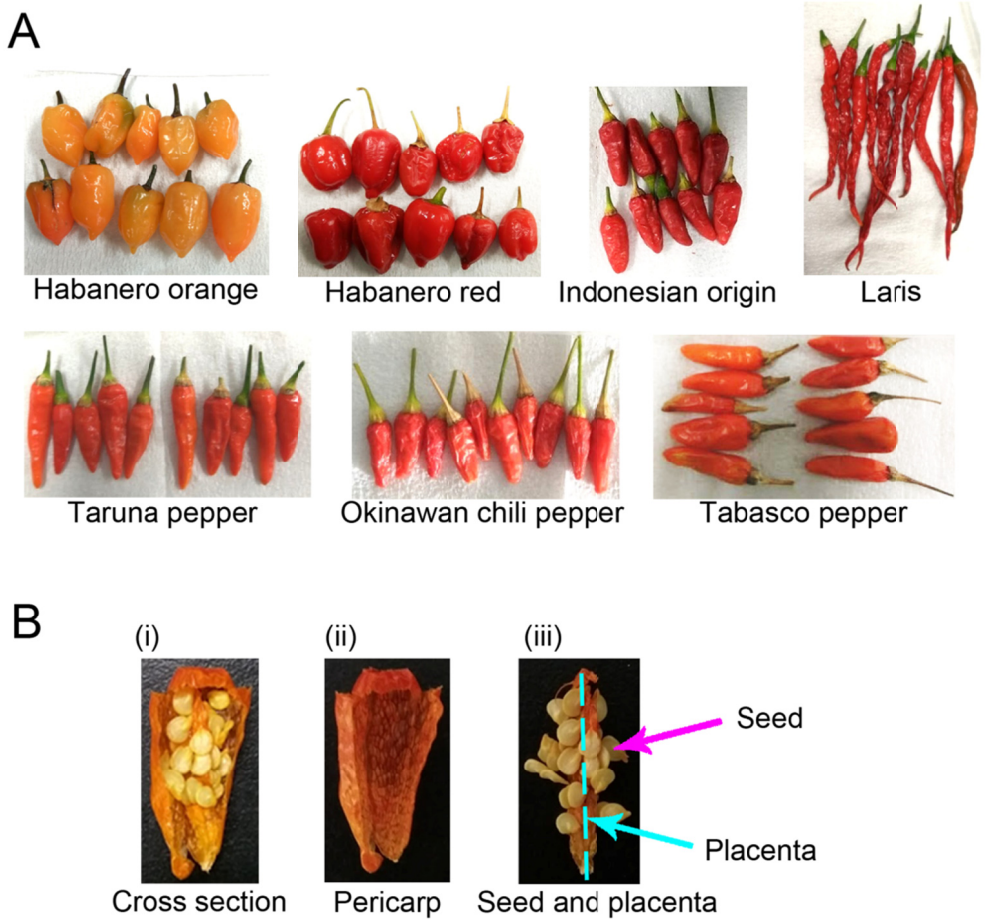


Figure 1. The photographs of peppers. (A) Representative photographs of the seven *Capsicum* varieties, including Habanero orange, Habanero red, Indonesian origin, Laris, Taruna pepper, Okinawan chili pepper, and Tabasco pepper, used in this study. (B) Sample preparation. Peppers were (i) carefully cut vertically, then separated into (ii) pericarps, and (iii) seeds and placenta

2.3 Quantitative Analysis of Pungent Components

Each dried sample (10 mg) was added to 1 mL acetonitrile, ultrasonically processed (ASU-6M, AS ONE) for 1 h, and centrifuged at 1,600 ×*g* for 10 min at 4 °C. The supernatants were used for analysis. After filtration through a 0.45-μm filter (Toyo Roshi Kaisha, Tokyo Japan), the samples were analyzed by high-pressure liquid chromatography (HPLC; Extrema, Jasco, Tokyo, Japan). Individual compounds were identified according to the retention times from highly selective spectral data generated using an ultraviolet-visible adsorption detector (UV-4075, Jasco) and a photodiode array detector (MD-4010, Jasco). The conditions for HPLC analysis were as follows: the C18 reversed-phase column (TSKgel ODS-100Z, 5 μm, 4.6 mm I.D. × 150 mm, Tosoh, Tokyo, Japan) and guard column (TSKgel Guardgel ODS-100Z, 5 μm, 3.2 mm I.D. × 15 mm, Tosoh) were maintained at 40 °C, and detection was conducted at 280 nm. The mobile phase consisted of 1% acetic acid in water (A) and

acetonitrile (B). We used a gradient of 0 min with 50% solution B, and 0-18 min with a direct increase in solution B of up to 75%. The flow rate was 1.0 mL/min and the injection volume was 10 μL.

2.4 Measurement of the Changes in Pungent Component Contents in Leaves and Fruits Over Time

We monitored the changes in the levels of pungent components in the leaves and fruits using a total of nine peppers, *i.e.*, the seven *Satsuma-Capsicum* varieties and the two traditional tropical-origin control peppers. The leaves and fruits were collected from four trees per variety because the composition of the plant varies depending on the location within the field (center or periphery). In addition, because there are differences in the growth of leaves and fruits depending on the amount of direct sunlight and wind, the pepper tree was divided into its upper and lower sections, and three leaves or fruits were collected from each section. Seeds of Habanero orange and Tabasco peppers were sown on April 14 and 15, 2017, and the plants were transferred to a sandy soil field on June 16, 2017. Thirty days after planting, *i.e.*, as soon as the plants showed sufficient growth to allow collection of leaves without compromising growth, leaves were collected 1-2 times per week for a total of 32 times. Collection continued until December 23, after which collection became impossible because of withering and leaf dropping. Flowers (harvested between August 10 and 26, 2017) and fruits (harvested between August 12 and December 23, 2017) were also sampled and analyzed for pungent component concentrations. To attenuate the effects of individual differences arising from harvesting, a minimum of three leaves, flowers, or fruits were collected from the top and bottom of each plant. Prior to harvesting, the overall condition of the plants was measured to ascertain the state of growth. After acquiring images using a digital camera, length, width, and weight were recorded. Colors were measured with a color-difference meter (CR-20; Konica Minolta, Tokyo, Japan) at four measurement points per sample. Leaf color was expressed using a color code conversion tool (freeware Color Converter, W3Schools) that converted the L*a*b system to the RGB system. L* expresses brightness, with values near 0 representing brightness closer to white and values near to 100 representing brightness closer to black. For a*, negative values indicate a shift toward green and positive values indicate a shift toward red. For b*, negative values indicate a shift toward blue and positive values indicate a shift toward yellow.

All samples were then cut with scissors and added (1 g/sample) to 1 mL ethanol, and incubated at 4 °C for 1 week. The extracts were subjected to ultrasonic processing for 10 min and centrifuged at 1,600 ×*g* for 10 min at 4 °C. The supernatants were passed through a 0.45-μm filter and used for measurement. HPLC conditions were the same as those for the quantification of pungent components.

2.5 Measurement of the Antioxidant Activity

We used the DPPH method to measure the antioxidant activity (Garcia et al., 2012; Kedare & Singh, 2011). In brief, 50 mg of dried sample was placed in a microcentrifuge tube, and 2 mL of hexane/dichloromethane (1:1) solution was added. The mixture was vortexed, ultrasonically treated for 10 min, and centrifuged at 1,600 ×*g* and 4 °C for 10 min. One milliliter of each supernatant was vacuum-concentrated (VEC-260; Iwaki, Tokyo, Japan). We measured the antioxidant activity using a total of nine peppers (fruits), *i.e.*, seven commonly consumed *Satsuma-Capsicum* varieties and two control peppers. One milliliter of 50% ethanol was added to each concentrated sample, and the sample was vortexed and ultrasonically treated until the concentrate was dissolved. Then, the sample solution (50 μL/well) was added to a 96-well microplate, and 50% ethanol was added to each well, as necessary. Next, 50 μL of an 800-μM DPPH solution was added to the sample solution and the plate was incubated at 25 °C for 20 min in the dark. The absorbance was measured at 540 nm with a microplate reader (Infinite 200 PRO; Tecan, Männedorf, Switzerland). A calibration curve was generated using the reference standard compound Trolox with a correlation coefficient of $R^2 = 0.9983$. Each experiment was conducted in quadruplicate. The DPPH scavenging effect was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = [(A0 - A1)/A0] \times 100 \quad (1)$$

where, A0 is the absorbance of the control reaction and A1 is the absorbance of the sample or standard.

2.6 NO Quantification Using a Modified Griess Method

We quantified the NO concentration using a total of nine peppers (fruits), *i.e.*, the seven commonly consumed *Satsuma*-Capsicum varieties and the two control peppers. Samples were extracted as described above. Typically, NO_2^- is measured using the Griess method (Bryan & Grisham, 2007; Schulz et al., 1999; Tsikas, 2007). NO has a short half-life, is unstable, and is hydrolyzed to NO_2^- and NO_3^-. When NO_3^- is reduced to NO_2^- using NO_3^- reductase and the total NO_2^- concentration is measured, the amount of NO can be indirectly measured. The 2,3-diaminonapthalene (DAN) fluorescence assay (Hu et al., 2014) was developed more recently; it is a NO_2^- assay with higher sensitivity than the Griess method. NO_2^- reacts with DAN under acidic conditions to form the

fluorescent adduct naphthalene triazole. Hence, we quantified the product by measuring the fluorescence intensity using a microplate reader. Human VECs were seeded in 96-well plates at 5.0×10^4 cells/mL and in HuMedia-EG2 (Kurabo, Osaka, Japan) supplemented with 2% fetal bovine serum and grown in a 5% CO_2 incubator (MCO-5AC, Panasonic, Osaka, Japan). When the cells reached 80% confluence, they were incubated in medium (100 μL) with or without pepper extract (15 μg/mL) for an additional 12-h period. Culture supernatants were collected by centrifugation at 1,000 ×*g* and 25 °C for 15 min and reduced with nitrate reductase and the respective enzyme cofactors (iron, molybdenum, and cytochrome) at 37 °C for 30 min. This was followed by a 15-min incubation with DAN. Fluorescence intensity was measured at an excitation of 360 nm and an emission of 450 nm with a microplate reader. The concentration of NO per sample was calculated by transforming raw data using a calibration curve (correlation coefficient $R^2 = 0.9905$) prepared with the $NaNO_2$ standard solution. The results are expressed as relative values normalized to the control value set at 1.

2.7 Statistical Analyses

Quantitative analysis for pungent components was repeated four times independently. Significant differences among groups were assessed using Student's *t*-tests and analysis of variance (ANOVA) as apt. Data are represented as the mean±standard deviation (SD). $P < 0.05$ was considered statistically significant.

3. Results and Discussion

The capsaicinoid contents of pericarp and s/p in the seven *Satsuma-Capsicum* varieties grown in sandy soil are shown in Figure 1. Calibration curves for capsaicin and dihydrocapsaicin were obtained for each standard. The correlation coefficients were $R^2 > 0.9998$ (capsaicin) and $R^2 > 0.9996$ (dihydrocapsaicin). Chromatograms showed retention times of 9.1 min for capsaicin and 12.5 min for dihydrocapsaicin. The pungent component contents per gram of pericarp and s/p were quantified (Figure 2). The s/p from *Satsuma*-Habanero orange, *Satsuma*-Taruna, and *Satsuma*-Tabasco peppers contained higher concentrations of pungent components than the pericarps. Previous studies have reported that pungent components migrate and disperse from the placenta to the pericarp (Canto-Flick et al., 2018; Pandhair & Sharma, 2008; Taira et al., 2012). Thus, it is possible that pungent components in *Satsuma*-Habanero red, *Satsuma*-Indonesian origin, *Satsuma*-Okinawan chili peppers, and Habanero orange tropical-origin control peppers made a transition faster than those in other cultivars. Furthermore, s/p from *Satsuma-Capsicum* peppers, including Habanero orange and Tabasco pepper, contained higher concentrations of capsaicinoids than those from tropical-origin control peppers. On the other hand, the concentrations in the pericarps were similar in both groups. Further, pungent components were not detected in any of the leaf or flower samples from Habanero orange or Tabasco peppers. It has been reported that the amount of capsaicin is affected by salt, water, and drought stress (Arrowsmith et al., 2012; Phimchan et al., 2012; Sung et al., 2005). Thus, it is conceivable that peppers cultivated under nutritionally adverse growing conditions contained higher levels of capsaicinoids in the s/p. Pepper cultivation under adverse conditions is suitable for increasing the amount of capsaicinoids. On the other hand, pungent components may be controlled by peppers cultivated under nutrient-rich and mild conditions.

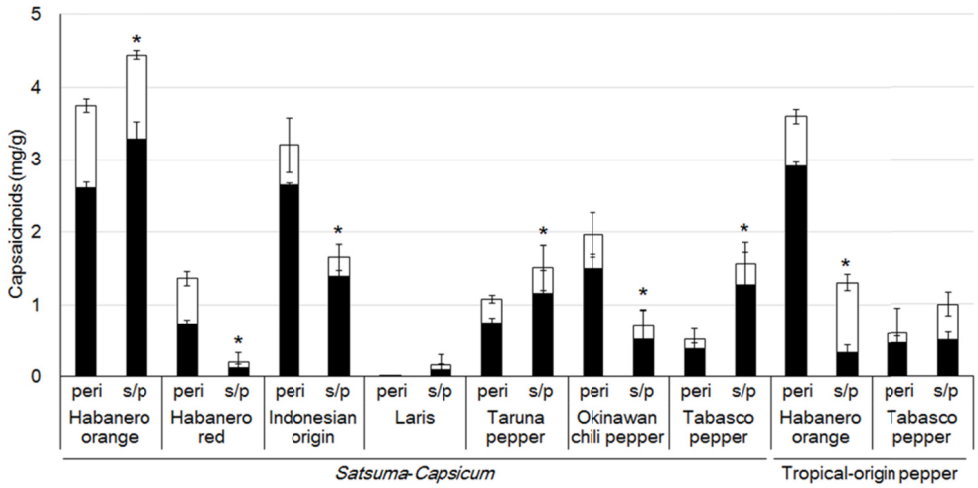


Figure 2. Quantification of capsaicinoids in *Satsuma-Capsicum* and tropical-origin peppers. Black bars represent capsaicin and white bars represent dihydrocapsaicin. Peri, pericarps; s/p, seeds and placenta. **P* < 0.05 versus total capsaicinoids in pericarps from the same pepper

The lower leaves from Habanero orange (Table 1) and Tabasco pepper (Table 2) plants were deeper in color and larger than the top leaves. In December, when the temperature rapidly decreases, leaves shriveled and turned yellow, and the fruits withered. After bearing fruits, the leaf length exceeded 10 cm. However, pungent components were not detected in any leaf or flower samples. Pungent compounds in fruits reached maximum concentrations 136 days after planting for Habanero orange (86 days after flowering and fruiting; collection No. 18), and 113 days after planting for Tabasco pepper (63 days after flowering and fruiting; collection No. 21). Thus, the peak point varied between cultivars (Figure 3). Notably, *Satsuma*-*Capsicum* had kept the b* value indicating low yellowness (Tables 1 and 2) and was slower to wither than tropical-origin control peppers. As a result, it could be harvested for about 60 days longer in sandy soil. This is a very important industrial advantage. The withered fruits also contained pungent components. We did not identify any correlation between pungent component contents in the fruits and leaf or fruit color. That is, to collect the fruits at an optimal time point, it was necessary to consider the time between planting, flowering, and fruiting, rather than simply observing visible changes.

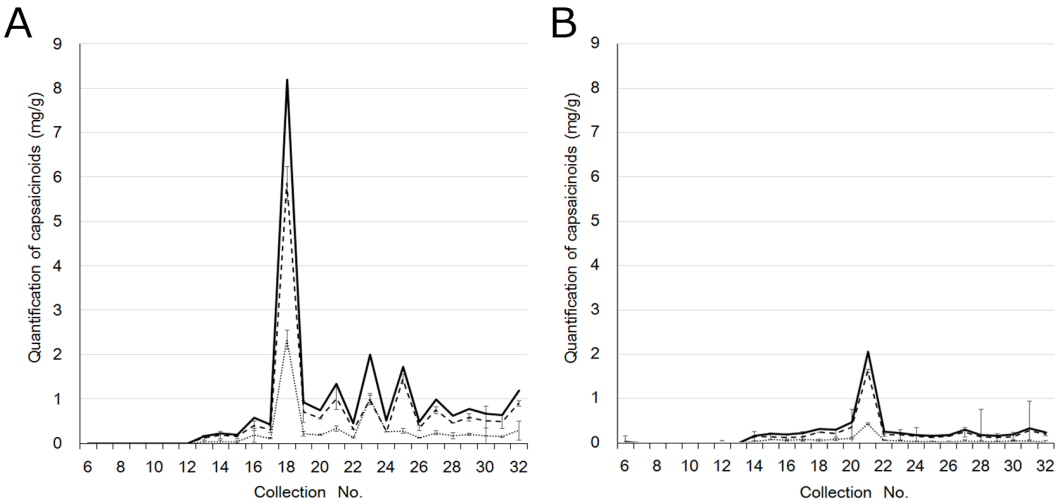
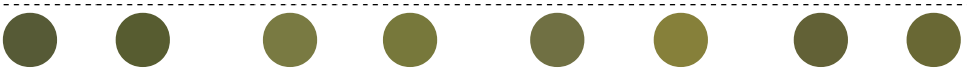


Figure 3. Changes over time in the concentrations of capsaicin and dihydrocapsaicin in (A) *Satsuma*-Habanero orange and (B) *Satsuma*-Tabasco pepper fruits. Dashed lines, capsaicin; dotted lines, dihydrocapsaicin; solid lines, total capsaicin and dihydrocapsaicin

Long-time consumption of capsaicin promotes fat reduction in humans (Arent et al., 2018; Leung, 2014) and the induction of skeletal muscle hypertrophy (Ito et al., 2013), and potentially reduces obesity (Almeida et al., 2014; Mun et al., 2014). These effects may be caused by capsaicin, a vanilloid belonging to the vanillyl group. Capsaicin potentially stimulates TRPV1, a receptor activation channel, by binding to vanilloid receptors; this could promote lipolysis and generate heat (Saito & Yoneshiro, 2013; Saito, 2015; Varghese et al., 2017; Wang et al., 2017). These biofunctions of capsaicinoids are expected to indorse the use of peppers cultivated under adverse conditions.

Table 1. Leaf characteristics during the growth period of *Satsuma*-Habanero orange peppers

Collection #	1		2		3		4	
Date	7/15/2017		7/22/2017		7/29/2017		8/5/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	5.7	5.6	7.5	6.8	4.7	6.6	6.9	8.1
Width (cm)	3.3	3.1	3.6	4.1	2.2	3.4	3.3	4.8
Weight (g)	0.246	0.270	0.361	0.483	0.136	0.377	0.317	0.735
L*	46.02±1.9	49.40±2.3	47.39±1.5	47.60±2.9	36.53±1.4	39.13±0.6	36.23±1.4	35.02±2.9
a*	-9.08±0.3	-10.09±0.2	-10.12±0.7	-10.37±0.5	-7.28±0.3	-8.75±0.3	-7.64±0.3	-8.42±0.9
b*	24.18±1.7	27.85±3.2	22.77±3.4	24.88±3.8	16.84±1.6	23.05±0.9	18.03±1.3	19.50±3.4
Color								

Collection #	5		6		7		8	
Date	8/10/2017		8/12/2017		8/17/2017		8/19/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	6.3	9.3	5.7	9.0	7.3	11.2	5.8	12.5
Width (cm)	2.7	4.3	2.8	4.5	3.3	6.0	2.7	6.6
Weight (g)	0.203	0.582	0.188	0.527	0.303	0.876	0.172	1.023
L*	42.75±1.0	45.53±3.1	35.68±1.2	33.37±1.2	36.32±1.7	34.03±1.1	35.43±0.9	32.45±1.7
a*	-8.55±0.6	-10.08±0.5	-7.82±0.4	-8.49±0.2	-7.67±0.3	-8.13±0.2	-7.18±0.2	-7.13±0.1
b*	18.58±2.1	24.85±3.6	17.03±1.3	18.49±0.9	19.79±1.2	17.78±1.2	17.22±1.4	15.53±1.1
Color								
Collection #	9		10		11		12	
Date	8/23/2017		8/26/2017		8/30/2017		9/2/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	5.7	11.4	5.9	4.5	5.5	10.4	5.7	12.0
Width (cm)	2.8	6.1	2.7	2.3	2.4	4.5	2.6	6.3
Weight (g)	0.174	0.922	0.153	0.781	0.162	0.536	0.177	0.994
L*	39.72±1.6	34.05±0.4	44.60±1.9	42.33±1.1	39.88±2.1	34.03±0.2	38.87±1.9	32.78±1.1
a*	-8.09±0.5	-7.88±0.1	-9.49±0.4	-10.00±0.2	-7.47±0.7	-8.28±0.1	-8.25±0.4	-8.55±0.2
b*	21.00±2.0	17.88±0.8	23.83±1.6	23.68±0.8	23.37±3.2	19.08±0.4	23.12±1.2	18.18±1.5
Color								
Collection #	13		14		15		16	
Date	9/6/2017		9/13/2017		9/16/2017		9/23/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	4.6	13.1	5.2	14.0	4.8	12.6	5.6	11.9
Width (cm)	2.2	7.2	2.5	6.7	2.1	6.0	2.2	5.7
Weight (g)	0.122	1.208	0.165	1.135	0.117	0.864	0.164	0.841
L*	36.90±1.4	32.45±0.6	39.60±1.9	32.13±0.9	38.73±1.7	36.43±0.3	39.25±1.4	35.28±1.2
a*	-8.54±0.3	-8.18±0.2	-8.85±0.3	-8.75±0.2	-8.93±0.6	-9.35±0.3	-8.47±0.3	-9.28±0.3
b*	21.09±0.7	16.38±0.8	22.68±1.3	18.18±1.1	18.73±2.3	21.85±0.7	20.58±1.3	20.73±1.2
Color								
Collection #	17		18		19		20	
Date	9/30/2017		10/7/2017		10/14/2017		10/21/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	4.5	12.5	5.5	13.5	6.2	14.0	5.4	9.6
Width (cm)	2.1	6.3	2.3	6.5	2.5	6.5	2.3	5.0
Weight (g)	0.108	0.963	0.164	1.290	0.181	1.215	0.153	0.620
L*	39.19±1.2	33.10±0.2	38.62±1.4	33.13±1.0	39.07±1.5	35.73±0.8	40.02±4.3	37.28±0.5
a*	-7.84±0.2	-8.23±0.1	-7.88±0.2	-8.50±0.0	-8.20±0.2	-8.68±0.2	-8.20±1.0	-9.38±0.2
b*	21.23±0.8	16.60±0.2	18.05±1.6	16.95±0.6	20.77±1.5	19.38±1.3	21.09±6.0	21.88±0.6
Color								
Collection #	21		22		23		24	
Date	10/30/2017		11/4/2017		11/9/2017		11/15/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	5.1	13.3	5.1	10.9	6.0	11.1	4.7	10.7
Width (cm)	2.0	6.7	2.7	5.9	2.5	5.6	2.1	5.0
Weight (g)	0.14	1.122	0.134	0.895	0.176	0.701	0.123	0.758
L*	37.24±1.0	37.49±1.0	49.85±2.7	49.20±4.3	46.55±3.1	52.73±1.8	40.28±3.3	43.04±3.4
a*	-8.25±0.2	-9.38±0.1	-9.42±0.7	-10.17±0.3	-7.75±0.5	-7.58±1.0	-6.48±0.5	-7.92±0.7
b*	20.59±1.0	24.08±1.3	30.58±4.6	33.18±6.1	25.27±4.6	38.05±2.7	24.87±4.5	29.39±5.5
Color								

Collection #	25		26		27		28	
Date	11/18/2017		11/25/2017		12/2/2017		12/6/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	5.3	10.6	5.1	11.5	7.0	11.8	5.2	8.9
Width (cm)	2.2	4.8	2.1	5.7	2.6	5.3	2.1	4.6
Weight (g)	0.171	0.599	0.155	0.838	0.253	0.817	0.154	0.467
L*	41.18±1.6	43.92±1.5	42.20±6.6	42.25±2.1	45.75±1.2	42.78±3.0	41.35±1.7	48.28±4.0
a*	-6.77±0.9	-6.80±0.9	-5.27±0.6	-7.03±0.7	-4.68±0.8	-6.58±2.1	-3.42±1.7	-2.99±1.2
b*	21.88±4.6	32.59±2.8	22.08±0.8	31.40±3.1	29.08±4.8	30.22±4.7	24.62±2.5	32.37±9.2
Color								
Collection #	29		30		31		32	
Date	12/9/2017		12/13/2017		12/20/2017		12/23/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	5.8	9	5.9	9.1	4.2	6.4	2.6	7.7
Width (cm)	2. 5	4.2	2.2	4.1	1.7	1.7	1.1	3.5
Weight (g)	0.201	0.504	0.153	0.439	0.05	0.142	0.023	0.190
L*	44.23±1.0	48.50±2.3	47.03±1.7	52.57±1.3	47.88±11.7	49.15±3.1	39.72±5.3	40.67±3.2
a*	-4.45±1.0	-0.58±1.7	-1.40±1.2	0.82±1.1	1.70±1.1	4.99±2.4	2.85±1.9	5.15±1.5
b*	29.88±2.7	37.99±3.0	30.33±4.5	40.40±2.6	17.8±9.1	24.89±2.4	10.24±4.3	26.13±2.7
Color								

Note. *Satsuma-Capsicum* leaves were gathered, and three leaves and fruits were collected from the top and bottom of the plant from four trees per strain. CIE L*a*b* color space values: L*, lightness; a*, green-red; b*, blue-yellow.

Table 2. Leaf characteristics during the growth period of *Satsuma*-Tabasco peppers

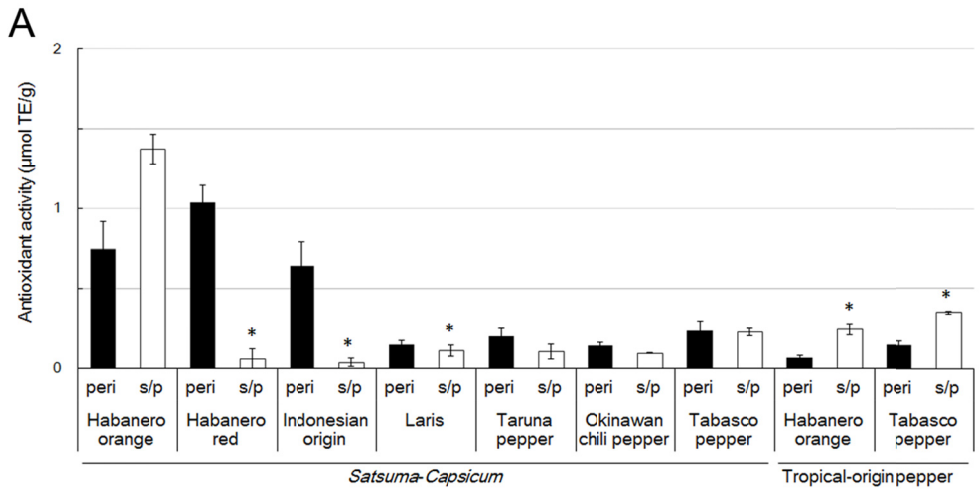
Collection #	1		2		3		4	
Date	7/15/2017		7/22/2017		7/29/2017		8/5/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	5.9	7.8	7.5	9.1	6.5	9.4	8.1	9.2
Width (cm)	3.1	4.2	3.2	4.8	3.0	4.9	3.8	4.5
Weight (g)	0.261	0.444	0.29	0.68	0.208	0.782	0.402	0.363
L*	45.37±4.3	45.69±2.5	43.80±2.1	41.68±1.4	36.52±1.2	34.24±1.1	35.52±0.9	34.05±0.6
a*	-8.79±0.7	-9.92±0.3	-9.32±0.3	-9.13±0.2	-7.20±0.3	-7.21±0.4	-7.58±0.1	-7.79±0.4
b*	22.45±1.1	28.18±2.7	22.14±1.7	21.58±0.9	18.83±0.9	17.40±1.4	19.13±0.6	17.20±1.6
Color								
Collection #	5		6		7		8	
Date	8/10/2017		8/12/2017		8/17/2017		8/19/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	7.0	9.4	6.2	9.6	7.5	9.1	5.8	12.1
Width (cm)	3.1	4.9	2.7	4.2	3.2	4.8	3.1	5.6
Weight (g)	0.268	0.838	0.213	0.658	0.290	0.680	0.200	1.026
L*	41.72±1.7	39.41±1.8	34.45±1.8	31.84±0.9	43.80±2.1	41.68±1.4	35.92±1.3	33.48±0.7
a*	-8.19±0.4	-8.35±1.1	-6.63±0.2	-7.44±0.4	-9.32±0.3	-9.13±0.2	-7.69±0.2	-7.18±0.3
b*	20.34±1.6	18.09±2.3	17.48±1.0	14.64±1.9	22.14±1.7	21.58±0.9	21.87±1.5	17.85±1.5
Color								

Collection #	9		10		11		12	
Date	8/23/2017		8/26/2017		8/30/2017		9/2/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	5.5	13.7	4.5	11.5	5.3	13.6	4.8	13.6
Width (cm)	3.0	6.4	2.3	5.5	2.5	6.1	2.5	6.0
Weight (g)	0.184	1.418	0.114	0.962	0.139	1.272	0.124	1.176
L*	35.60±2.4	32.13±1.8	45.23±2.5	41.79±0.8	36.07±1.4	34.18±1.1	38.07±1.6	35.05±1.1
a*	-6.90±0.2	-7.20±0.4	-9.58±0.5	-9.19±0.1	-7.37±0.2	-8.23±0.4	-7.50±0.3	-8.58±0.3
b*	20.43±1.7	14.98±2.6	26.20±1.6	22.24±0.6	23.05±1.4	18.75±1.9	22.73±1.3	19.98±1.6
Color								
Collection #	13		14		15		16	
Date	9/6/2017		9/13/2017		9/16/2017		9/23/2017	
Position	Top	Bottom	Top	Top	Top	Bottom	Top	Bottom
Length (cm)	4.7	13.9	5.4	4.9	4.9	15.2	4.9	12.1
Width (cm)	2.3	5.8	2.5	2.4	2.4	6.7	2.4	5.9
Weight (g)	0.111	1.405	0.133	0.107	0.107	1.566	0.107	1.317
L*	36.97±1.4	33.05±0.7	36.74±1.7	35.02±3.3	35.02±3.3	37.60±2.3	35.02±3.3	36.40±0.4
a*	-8.14±0.2	-7.33±0.2	-8.73±0.2	-7.98±0.9	-7.98±0.9	-9.13±0.3	-7.98±0.9	-8.95±0.1
b*	21.40±1.7	15.33±0.7	22.69±1.2	19.20±3.7	19.20±3.7	22.33±2.8	19.20±3.7	21.08±0.6
Color								
Collection #	17		18		19		20	
Date	9/30/2017		10/7/2017		10/14/2017		10/21/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	4.6	12.7	4.7	11.5	4.1	13.6	5.3	11.5
Width (cm)	2.4	6	2.5	5.6	2.3	6.0	2.5	5.0
Weight (g)	0.124	1.213	0.142	0.948	0.103	1.348	0.192	0.504
L*	34.73±1.3	39.63±0.8	34.53±1.8	40.18±1.9	36.99±0.6	41.55±2.4	32.92±1.8	33.35±0.2
a*	-7.37±0.4	-9.00±0.4	-7.32±0.5	-9.20±0.3	-7.77±0.1	-8.55±0.5	-6.80±0.6	-8.00±0.2
b*	19.27±0.9	24.28±2.7	16.44±2.1	24.98±2.6	18.59±0.6	26.68±3.3	13.80±2.2	15.58±0.3
Color								
Collection #	21		22		23		24	
Date	10/30/2017		11/4/2017		11/9/2017		11/15/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	4.5	11.2	5.1	10.1	5.4	11.2	4.0	10.1
Width (cm)	2.4	5.7	2.7	5.6	2.6	5.3	2.2	4.5
Weight (g)	0.144	0.926	0.213	0.749	0.179	0.850	0.149	0.571
L*	35.37±5.4	33.72±1.7	38.84±0.9	41.84±4.0	38.48±0.7	48.14±1.5	31.47±0.8	34.90±2.8
a*	-6.59±1.0	-7.87±0.4	-6.83±0.7	-8.55±0.7	-6.87±0.3	-9.50±0.7	-5.04±0.1	-7.54±0.8
b*	18.60±8.9	16.42±1.8	13.98±1.7	18.08±4.3	13.93±0.9	29.45±2.4	12.43±0.7	17.88±4.8
Color								
Collection #	25		26		27		28	
Date	11/18/2017		11/25/2017		12/2/2017		12/6/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	4.6	10.3	4.3	10.3	5.4	10.4	5.4	11.2
Width (cm)	2.6	5.1	2.5	5.0	3.1	5.3	2.6	5.3
Weight (g)	0.189	0.786	0.167	0.626	0.206	0.681	0.179	0.850
L*	34.65±1.4	38.37±3.3	32.73±1.6	33.34±3.3	34.65±1.9	37.19±3.2	38.48±0.7	48.14±1.5
a*	-5.39±0.7	-8.23±0.4	-5.04±0.6	-7.17±0.8	-5.90±0.6	-6.92±1.2	-6.87±0.3	-9.50±0.7
b*	15.97±2.0	23.00±4.8	14.17±3.2	15.00±4.5	17.33±2.7	21.42±4.8	13.93±0.9	29.45±2.4
Color								

Collection #	29		30		31		32	
Date	12/9/2017		12/13/2017		12/20/2017		12/23/2017	
Position	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Length (cm)	4.7	12.0	5.0	8.5	4.9	7.0	4.7	5.3
Width (cm)	2.5	6.0	2.4	4.3	3.0	3.7	2.3	2.5
Weight (g)	0.169	0.957	0.175	0.494	0.181	0.083	0.106	0.080
L*	35.84±1.1	42.28±2.4	34.64±2.2	42.63±3.7	35.30±2.8	33.53±3.2	32.43±1.9	31.50±4.8
a*	-4.95±0.5	-5.94±2.8	-4.60±1.3	-4.77±0.7	-2.27±1.8	-2.48±3.1	-1.64±2.2	-2.40±1.8
b*	19.04±1.4	28.30±4.4	16.52±3.9	29.87±5.4	15.70±3.6	15.75±3.1	12.99±3.1	14.15±4.6
Color								

Note. *Satsuma-Capsicum* leaves were gathered and three leaves and fruits each from the top and bottom of the plant were collected from four trees per strain. CIE L*a*b* color space values: L*, lightness; a*, green-red; b*, blue-yellow.

Extracts of all *Satsuma-Capsicum* showed antioxidant activity *in vitro* (Figure 4A). The antioxidant capacity of s/p, but not the pericarp, of *Satsuma*-Habanero orange was significantly higher than that of tropical-origin Habanero orange (control), which indicates the great advantages of the utilization of peppers cultivated under adverse conditions. Furthermore, the antioxidant activity of the pericarp of Habanero red and Indonesian origin peppers was significantly higher than that of the s/p. There was no correlation between antioxidant activity and pungent compound content (R^2 = 0.4579). In addition to its pungent components, pepper has many functional components, including carotenoid pigments, capsanthin, and α-tocopherol. Therefore, we reason that the antioxidant properties may be caused by interactions between the liposoluble carotenoid pigments capsanthin and α-tocopherol, rather than the pungent components (Fernández-García et al., 2016; Sun et al., 2007).



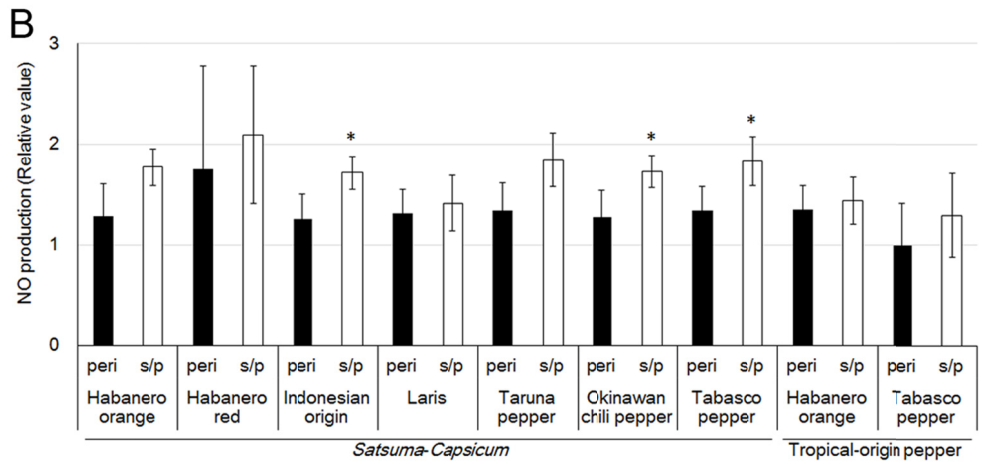


Figure 4. The antioxidant activity and the effect of peppers on vascular endothelial function. (A) Antioxidant activity of and (B) the level of nitric oxide (NO) production in vascular endothelial cells induced by *Satsuma-Capsicum* and tropical-origin pepper extracts. Antioxidant activity is expressed as Trolox equivalent. The total NO concentration is calculated indirectly from NO_2^-concentrations (including NO_2^- reduced from NO_3^- using NO_3^- reductase). Data were normalized to the control level. Peri, pericarps; s/p, seeds and placenta. *$P <$ 0.05 versus pericarps

Pepper and paprika varieties are widely considered to promote health, as they exhibit high antioxidant activity and possess properties that limit cancer cell proliferation (Daood et al., 1996; Markus et al., 1999; Škrovánková et al., 2017). Because paprika contains only trace amounts of capsaicin and dihydrocapsaicin, these properties were thought to result from the activity of capsanthin or carotenoid pigments. Moreover, peppers reportedly contain polyphenols with multiple biomodulatory functions (Materska & Perucka, 2005; Mokhtar et al., 2015; Oboh & Rocha, 2007). The *Satsuma-Capsicum* cultivated under adverse conditions exhibited significant antioxidative activity, which may be due to polyphenols.

Finally, we investigated whether *Satsuma-Capsicum* would promote NO production in VECs at concentrations similar to those of tropical-origin control peppers to confirm their bioregulatory effect on vascular function. The results indicate that all pepper extracts increased NO production, with no significant differences between pericarp and s/p extracts. In addition, the effects of *Satsuma-Capsicum* extracts on NO production were similar to those of the tropical-origin control pepper extracts (Figure 4B). This suggests that the increase of NO production that leads to improved vascular endothelial function is not related to the amount of capsaicinoid.

4. Conclusion

Peppers cultivated under adverse conditions contained higher levels of capsaicinoids than those contained in peppers cultivated in nutrient-rich conditions. In particular, since the s/p contained high levels of capsaicinoids, it is conceivable that these are not transferred to the pericarp, accumulating in the seeds and placenta, when cultivated under adverse conditions. The withered fruits also contained pungent components. We did not identify any correlation between pungent component contents in the fruits and leaf or fruit color. To collect the fruits at an optimal time point, it was necessary to consider the time between planting, flowering, and fruiting, rather than simply observing visible changes. All *Satsuma-Capsicum* plants cultivated under adverse conditions in southern Japan showed higher antioxidant activity than control peppers. We reason that the antioxidant properties may be caused by interactions between the liposoluble carotenoid pigments capsanthin and α-tocopherol, rather than the pungent components. In addition, their effects on NO production were similar to control pepper extracts; thus, *Satsuma-Capsicum* potentially improves vascular endothelial function. This suggests that the increase of NO production that leads to improved vascular endothelial function is not related to the amount of capsaicinoid. Finally, the *Satsuma-Capsicum* cultivated in this study were slower to spoil than peppers cultivated under nutrient-rich conditions and could be harvested over a longer period of time. Our results suggest that adverse condition-cultivated Capsicum are superior to nutrient-rich condition-cultivated peppers and may be valuable for future commercial development.

Acknowledgements

This work was supported by JSPS KAKENHI (17K07795) and a Project of the Bio-Oriented Technology Research Advancement Institution, NARO. We would like to thank Dr. Fumio Yagi for technical advice. We would also like to thank Dr. Jun-Ichi Sakagami and Mr. Kenta Komori for providing *Capsicum* samples.

References

Almeida, M. A., Nadal, J. M., Grassiolli, S., Paludo, K. S., Zawadzki, S. F., Cruz, L., … Farago, P. V. (2014). Enhanced gastric tolerability and improved anti-obesity effect of capsaicinoids-loaded PCL microparticles. *Material Science and Engineering, 40*, 345-356. https://doi.org/10.1016/j.msec.2014.03.049

Arent, S. M., Walker, A. J., Pellegrino, J. K., Sanders, D. J., McFadden, B. A., Ziegenfuss, T. N., & Lopez, H. L. (2018). The combined effects of exercise, diet, and a multi-ingredient dietary supplement on body composition and adipokine changes in overweight adults. *The Journal of the American College of Nutrition, 37*, 111-120. https://doi.org/10.1080/07315724.2017.1368039

Arrowsmith, S., Egan, T. P., Meekins, J. F., Powers, D., & Metcalfe, M. (2012). Effects of salt stress on capsaicin content, growth, and fluorescence in a Jalapeño cultivar of *Capsicum annuum* (Solanaceae). *Bios, 83*, 1-7. https://doi.org/10.1893/0005-3155-83.1.1

Banedjschafie, S., & Durner, W. (2015). Water retention properties of a sandy soil with superabsorbent polymers as affected by aging and water quality. *Journal of Plant Nutrition and Soil Science, 178*, 798-806. https://doi.org/10.1002/jpln.201500128

Bryan, N. S., & Grisham, M. B. (2007). Methods to detect nitric oxide and its metabolites in biological samples. *Free Radical Biology & Medicine, 43*, 645-657. https://doi.org/10.1016/j.freeradbiomed.2007.04.026

Canto-Flick, A., Balam-Uc, E., Bello-Bello, J. J., Lecona-Guzmán, C., Solís-Marroquín, D., Avilés-Viñas, S., & Santana-Buzzy, N. (2008). Capsaicinoids content in Habanero pepper (*Capsicum chinense* Jacq.): Hottest known cultivars. *HortScience, 43*, 1344-1349. https://doi.org/10.21273/HORTSCI.43.5.1344

Chularojmontri, L., Suwatronnakorn, M., & Wattanapitayakul, S. K. (2010). Influence of *Capsicum* extract and capsaicin on endothelial health. *Journal of the Medical Association of Thailand, 93*, S92-101.

Daood, H. G., Vinkler, M., Markus, F., Hebshi, E. A., & Biacs, P. A. (1996). Antioxidant vitamin content of spice red pepper (paprika) as affected by technological and varietal factors. *Food Chemistry, 55*, 365-372. https://doi.org/10.1016/0308-8146(95)00136-0

Fernández-García, E., Carvajal-Lérida, I., & Pérez-Gálvez, A. (2016). Carotenoids exclusively synthesized in red pepper (capsanthin and capsorubin) protect human dermal fibroblasts against UVB induced DNA damage. *Photochemical & Photobiological Science, 15*, 1204-1211. https://doi.org/10.1039/C6PP00134C

Garcia, E. J., Oldoni, T. L. C., Alencar, S. M. D., Reis, A., Loguercio, A. D., & Grande, R. H. (2012). Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. *Brazilian Dental Journal, 23*, 22-27. https://doi.org/10.1590/S0103-64402012000100004

Hu, T. M., Chiu, S. J., & Hsu, Y. M. (2014). Nitroxidative chemistry interferes with fluorescent probe chemistry: implications for nitric oxide detection using 2,3-diaminonaphthalene. *Biochemical and Biophysical Research Communications, 451*, 196-201. https://doi.org/10.1016/j.bbrc.2014.07.097

Ito, N., Ruegg, U. T., Kudo, A., Miyagoe-Suzuki, Y., & Takeda, S. (2013). Capsaicin mimics mechanical load-induced intracellular signaling events: Involvement of TRPV1-mediated calcium signaling in induction of skeletal muscle hypertrophy. *Channels, 7*, 221-224. https://doi.org/10.4161/chan.24583

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology, 48*, 412-422. https://doi.org/10.1007/s13197-011-0251-1

Kuroda, R., Kazumura, K., Ushikata, M., Minami, Y., & Kajiya, K. (2018). Elucidating the improvement in vascular endothelial function of Sakurajima Daikon and its mechanism of action: A comparative study with *Raphanus sativus*. *Journal of Agricultural Food Chemistry, 66*, 8714-8721. https://doi.org/10.1021/acs.jafc.8b01750

Leung, F. W. (2014). Capsaicin as an anti-obesity drug. *Progress in Drug Research, 68*, 171-179. https://doi.org/10.1007/978-3-0348-0828-6_7

Márkus, F., Daood, H. G., Kapitány, J., & Biacs, P. A. (1999). Change in the carotenoid and antioxidant content of spice red pepper (paprika) as a function of ripening and some technological factors. *Journal of Agricultural Food Chemistry, 47*, 100-107. https://doi.org/10.1021/jf980485z

Materska, M., & Perucka, I. (2005). Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). *Journal of Agricultural Food Chemistry, 53*, 1750-1756. https://doi.org/10.1021/jf035331k

McCarty, M. F., DiNicolantonio, J. J., & O'Keefe, J. H. (2015). Capsaicin may have important potential for promoting vascular and metabolic health. *Open Heart, 2*, e000262. https://doi.org/10.1136/openhrt-2015-000262

Mokhtar, M., Soukup, J., Donato, P., Cacciola, F., Dugo, P., Riazi, A., … Mondello, L. (2015). Determination of the polyphenolic content of a *Capsicum anuum* L. extract by liquid chromatography coupled to photodiode array and mass spectrometry detection and evaluation of its biological activity. *Journal of Separation Science, 38*, 171-178. https://doi.org/10.1002/jssc.201400993

Monforte-González, M., Guzmán-Antonio, A., Uuh-Chim, F., & Vázquez-Flota, F. (2010). Capsaicin accumulation is related to nitrate content in placentas of habanero peppers (*Capsicum chinense* Jacq.). *Journal of the Science of Food and Agriculture, 90*, 764-768. https://doi.org/10.1002/jsfa.3880

Mun, J. M., Ok, H. M., & Kwon, O. (2014). Corn gluten hydrolysate and capsaicin have complimentary actions on body weight reduction and lipid-related genes in diet-induced obese rats. *Nutrition Research, 34*, 458-465. https://doi.org/10.1016/j.nutres.2014.04.009

Ng, C. M., & Reuter, W. M. (2015). Analysis of capsaicin and dihydrocapsaicin in chili peppers using the PerkinElmer altus HPLC system with PDA detection. *PerkinElmer Food Nutrition Compendium* (pp. 67-71).

Oboh, G., & Rocha, J. B. T. (2007). Polyphenols in red pepper (*Capsicum annuum* var aviculare Tepin) and their protective effects on some pro-oxidant induced lipid peroxidation in brain and liver. *European Food Research and Technology, 225*, 239-247. https://doi.org/10.1007/s00217-006-0410-1

Othman, Z. A. A., Ahmed, Y. B. H., Habila, M. A., & Ghafar, A. A. (2011). Determination of capsaicin and dihydrocapsaicin in *Capsicum* fruit samples using high performance liquid chromatography. *Molecules, 16*, 8919-8929. https://doi.org/10.3390/molecules16108919

Pandhair, V., & Sharma, S. (2008). Accumulation of capsaicin in seed, pericarp and placenta of *Capsicum annuum* L fruit. *Journal of Plant Biochemistry and Biotechnology, 17*, 23-27. https://doi.org/10.1007/BF03263255

Phimchan, P., Techawongstien, S., Chanthai, S., & Bosland, P. W. (2012). Impact of drought stress on the accumulation of capsaicinoids in capsicum cultivars with different initial capsaicinoid Levels. *HortScience, 47*, 1204-1209. https://doi.org/10.21273/HORTSCI.47.9.1204

Reyes-Escogido, M. D. L., Gonzalez-Mondragon, E. G., & Vazquez-Tzompantzi, E. (2011). Chemical and pharmacological aspects of capsaicin. *Molecules, 16*, 1253-1270. https://doi.org/10.3390/molecules 16021253

Roper, M. M., Davies, S. L., Blacjwell, P. S., Hall, D. J. M., Bakker, D. M., Jongepier, R., & Ward, P. R. (2015). Management options for water-repellent soils in Australian dryland agriculture. *Soil Research, 53*, 786-806. https://doi.org/10.1071/SR14330

Saito, M. (2015). Capsaicin and related food ingredients reducing body fat through the activation of TRP and brown fat thermogenesis. *Advances in Food and Nutrition Research, 76*, 1-28. https://doi.org/10.1016/bs.afnr.2015.07.002

Saito, M., & Yoneshiro, T. (2013). Capsinoids and related food ingredients activating brown fat thermogenesis and reducing body fat in humans. *Current Opinion in Lipidology, 24*, 71-77. https://doi.org/10.1097/MOL.0b013e32835a4f40

Schulz, K., Kerber, S., & Kelm, M. (1999). Reevaluation of the Griess method for determining NO/NO_2^- in aqueous and protein-containing samples. *Nitric Oxide, 3*, 225-234. https://doi.org/10.1006/niox.1999.0226

Sim, K. H., & Sil, H. Y. (2008). Antioxidant activities of red pepper (*Capsicum annuum*) pericarp and seed extracts. *International Journal of Food Science & Technology, 43*, 1813-1823. https://doi.org/10.1111/j.1365-2621.2008.01715.x

Škrovánková, S., Mlček, J., Orsavová, J., Juríková, T., & Dřímalová, P. (2017). Polyphenols content and antioxidant activity of paprika and pepper spices. *Journal of Food Science, 11*, 52-57. https://doi.org/10.5219/695

Song, W., Derito, C. M., Liu, M. K., He, X., Dong, M., & Liu, R. H. (2010). Cellular antioxidant activity of common vegetables. *Journal of Agricultural Food Chemistry, 58*, 6621-6629. https://doi.org/10.1021/jf9035832

Sun, T., Xu, Z., Wu, C. T., Janes, M., Prinyawiwatkul, W., & No, H. K. (2007). Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). *Journal of Food Science, 72*, S98-102. https://doi.org/10.1111/j.1750-3841.2006.00245.x

Sung, Y. L., Chang, Y. Y., & Ting, N. L. (2005). Capsaicin biosynthesis in water-stressed hot pepper fruits. *Botanical Bulletin of Academia Sinica, 46*, 35-42. https://doi.org/10.7016/BBAS.200501.0035

Taira, S., Shimma, S., Osaka, I., Kaneko, D., Ichiyanagi, Y., Ikeda, R., … Komatsu, K. (2012). Mass spectrometry imaging of the capsaicin localization in the *Capsicum* fruits. *International Journal of Biotechnology for Wellness Industries, 1*, 61-65. https://doi.org/10.6000/1927-3037.2012.01.01.04

Tsikas, D. (2007). Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: Appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. *Journal of Chromatography B, 851*, 51-70. https://doi.org/10.1016/j.jchromb.2006.07.054

Varghese, S., Kubatka, P., Rodrigo, L., Gazdikova, K., Caprnda, M., Fedotova, J., … Büsselberg, D. (2017). Chili pepper as a body weight-loss food. *International Journal of Food Science and Nutrition, 68*, 392-401. https://doi.org/10.1080/09637486.2016.1258044

Wakamatsu, M., Yamanouchi, H., Sahara, H., Iwanaga, T., Kuroda, R., Yamamoto, A., … Kajiya, K. (2019). Catechin and caffeine contents in green tea at different harvest periods and their metabolism in miniature swine. *Food Science and Nutrition, 7*, 2769-2778. https://doi.org/10.1002/fsn3.1143

Wang, Y., Cui, L., Xu, H., Liu, S., Zhu, F., Yan, F., … Zhu, M. (2017). TRPV1 agonism inhibits endothelial cell inflammation via activation of eNOS/NO pathway. *Atherosclerosis, 260*, 13-19. https://doi.org/10.1016/j.atherosclerosis.2017.03.016

Zhang, Z. X., Zhao, S. N., Liu, G. F., Huang, Z. M., Cao, Z. M., Cheng, S. H., & Lin, S. S. (2016). Discovery of putative capsaicin biosynthetic genes by RNA-Seq and digital gene expression analysis of pepper. *Scientific Reports, 6*, 1-14. https://doi.org/10.1038/srep34121