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Original paper

Physical Properties, Flavor Characteristics and Antioxidant Capacity of Shimatogarashi (*Capsicum frutescens*)

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Physical properties, flavor characteristics and antioxidant properties of the red mature peppers of Shimatogarashi (*Capsicum frutescens*) were compared to Takanotsume peppers (*Capsicum annuum*) as the control. Compositions of organic acids and capsaicinoids differed between the peppers. Malic acid was the prevalent organic acid in Shimatogarashi, while citric acid was prevalent in Takanotsume. Shimatogarashi had higher capsaicin and dihydrocapsaicin contents compared to Takanotsume. In terms of volatile compound compositions, the prevalent compounds in Shimatogarashi and Takanotsume peppers were hexanal and 10*s*,11*s*-himachala-3(12),4-diene, respectively. Overall, Shimatogarashi peppers were brightly colored, highly pungent with a fresh and fruity aroma, while Takanotsume peppers were dark red, moderately pungent with a warm and herbaceous aroma. As suggested by their total phenolic content and ORAC, Shimatogarashi peppers showed higher antioxidant activity compared to Takanotsume peppers.

Keywords: chili pepper, flavor, antioxidant, *C. annuum*, *C. frutescens*

Introduction

Chili peppers (*Capsicum* spp.) are remarkable culinary ingredients because of their attractive color, spiciness and complex aroma, making them appealing components of cuisines throughout the world. Nowadays, these fruits are not only eaten fresh, but are also dehydrated or used in sauces, infusions and as additives. *Capsicum* fruits are not only a well known culinary ingredient, but researchers have also identified many health benefits, such as antioxidant, antianalgesic and antiobesity effects, related to their capsaicinoids (Luo *et al*., 2011).

Regarding consumer preference for chili peppers, several factors are taken into consideration, such as flavor, aroma, pungency and appearance. Flavor perception is affected by a combination of these factors, and which has the potential to increase appetite; thus, flavor additives in food products increase their value (Eggink *et al*., 2012).

Chili peppers are believed to originate in the tropical climates of South and Central America, and the Galapagos (Walsh and Hoot, 2001). Of the 25 species of the genus *Capsicum* investigated, Yamamoto (2013) reported that two species had been introduced to Japan: *C. annuum* and *C. frutescens*, known in Japan as Takanotsume and Shimatogarashi, respectively. Takanotsume is a commonly used pepper in mainland Japan, whereas Shimatogarashi is mostly cultivated in the Okinawa region of Japan. In this region, Shimatogarashi peppers are traditionally used in the uniquely flavored spice known as *koregusu*.

Flavor characteristics and food functional properties can be used to differentiate between cultivars and their place of origins, as

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well as to assess their authenticity. For example, in order to prevent fraud, the volatile composition of Brazilian chili peppers (*Capsicum* spp.) had been profiled. However, the compositional investigation of chili peppers is challenging owing to their edaphoclimatic nature and large genetic diversity (Junior *et al*. 2012). The flavor characteristics (volatile composition in particular) and functional properties (antioxidant activity in particular) of chili peppers, especially *C. annuum*, which includes Takanotsume, have been studied (Eggink *et al.,* 2012; Meckelmann *et al.,* 2013), as they are one of the best known and most widely used spice crops in the world. In contrast, *C. frutescens* domesticated in subtropical Asian regions, and particularly Shimatogarashi peppers from Okinawa, have not been extensively investigated. Thus, it is of great interest to determine the food properties of Shimatogarashi in terms of flavor characteristics and functional properties.

The aim of this study was to characterize the physical properties and flavor quality attributes, such as organic acid and capsaicinoid composition and volatile components, of Shimatogarashi compared to Takanotsume as the control. We also evaluated the functional properties of the peppers by analyzing total phenolic content and antioxidant activity using oxygen radical absorption capacity (ORAC) assay. This is the first report on the physical properties, flavor characteristics and antioxidant capacity of Shimatogarashi in Okinawa, Japan.

Materials and Methods

Plant materials Shimatogarashi (*C. frutescens*) seeds were obtained from Miyako Island, courtesy of the Okinawa Prefectural Agricultural Research Center. Takanotsume (*C. annuum*) seeds were purchased as commercially available seed packages. The plants were cultivated at the Subtropical Field Science Center, University of the Ryukyus, Okinawa, Japan. Germination was initiated by planting the seeds in a tray filled with pumice sand on March 21, 2013. Once emerged, the seedlings were provided with nutrient solution (NO_3-N: PO_4-P: K: Ca: Mg = 18.6: 5.1: 8.6: 8.2: 3.0 mg/L) and kept in a greenhouse for two months. Shimatogarashi and Takanotsume greenhouse-grown plants were then transplanted and cultivated in a field plot consisting of a 24 m × 1 m field of mixed soil (gray soil and dark-red soil, pH 6.84) with compost (N: P: K = 1.0: 2.3: 2.1%) until plants bore abundant fruit. Fruits were randomly picked from ten different plants and stored at −30℃ in a freezer until analysis, unless otherwise indicated.

Chemicals Standard organic acids (malic, citric and *L*-ascorbic acids) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Standard capsaicinoids (capsaicin and dihydrocapsaicin) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chemicals used as standards to identify volatile components were obtained from Tokyo Chemical Industry (Tokyo, Japan), Wako Pure Chemical Industries, Kanto Chemical Industry (Tokyo, Japan), ACROS (Morris Plains, NJ, USA), Fluka (Buschs, Switzerland) and Sigma-Aldrich. 2,2'-Azobis(2-methylpropionamidine)dihydrochloride (AAPH) and gallic acid were obtained from Wako Pure Chemical Industries. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Calbiochem (San Diego, CA, USA). Folin-Ciocalteu reagent was purchased from Nacalai Tesque (Kyoto, Japan). All other reagents were of analytical grade.

Analysis of surface color Reflective color information was obtained using a handy NF 333 spectrophotometer (Nippon Denshoku Industries Co., Tokyo, Japan) with an 8 mm diameter sensor and standard calibration plate (No. 99067). The observed color was mapped in the CIE color space using the L^* a^* b^* coordinates.

Analysis of organic acids Organic acids, including malic and citric acids, were extracted from the fruit as follows. Briefly, sliced fruit (5 g) was mixed in ultra-pure water (20 mL) using a homogenizer (Ultra Turrax T25 basic, Labortechnik, Selangor, Malaysia), and the mixture was then centrifuged (CR20 GIII, Hitachi, Tokyo, Japan) at 32300 × *g* and 4℃ for 30 min. The supernatant was collected and filtered through a Sep-Pak C18 cartridge (Waters, Milford, MA, USA) and an Advantec 0.45 μm cellulose acetate membrane filter (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The organic acids in the filtrate were analysed by high-performance liquid chromatography (HPLC) according to the method of Ji *et al.* (2006), with slight modifications. A Shim-pack SCR-102H column (300 mm × 8 mm i.d., Shimadzu Corp., Kyoto, Japan) connected to a guard column (50 mm × 6 mm i.d.) was employed. Two Shimadzu LC-10 AD pumps were used to flow the mobile phase containing 5 mM *p*-toluenesulfonic acid and post-column detection reagent containing 5 mM *p*-toluenesulfonic acid, 100 μM ethylenediaminetetraacetic acid (EDTA) disodium salt, and 20 mM Bis-Tris buffer in isocratic mode at a flow rate of 0.7 mL/min. The mobile phase and post-column detection solvent were streamed to a post-column reactor and mixed at a ratio of 1:1 before detection with a Shimadzu CDD-6A conductivity detector. The column, guard column and post-column reactor were maintained at a constant temperature of 40℃ using a Shimadzu CTO-10 AC oven, and the injection volume was 10 μL. The concentrations of citric and malic acids were calibrated by plotting peak area against concentration for the respective acid standards and were expressed as mg/100 g-fresh weight (FW). All assays were performed in triplicate.

L-Ascorbic acid concentration was determined by the method of Topuz and Ozdemir (2007), with slight modifications. Briefly, sliced fruit (5 g) was mixed in 20 mL of 30 g/L metaphosphoric acid aqueous solution including 1 μM EDTA, and 10 μM diethyldithiocarbamic acid using a homogenizer, and the mixture was then centrifuged at 32300 × *g* at 4℃ for 30 min. The supernatant was collected and filtered through a Sep-Pak C18 cartridge and a 0.45 μm cellulose acetate membrane filter. Ascorbic acid in the filtrate was analysed by HPLC with a Cosmosil 5C_{18}-

AR-II column (250 mm × 4.6 mm i.d., Nacalai Tesque) and Shimadzu SPD-M20A diode array detector using a mobile phase of 0.2 M potassium dihydrogen phosphate solution (pH 2.2 adjusted with *o*-phosphoric acid). The flow rate and oven temperature were 0.7 mL/min and 28℃, respectively. The injection volume of samples and standards was 5 µL. The ascorbic acid peak was monitored at 254 nm and its concentration was calibrated by plotting peak area against standard concentrations, which was expressed as mg/100 g-FW. All assays were performed in triplicate.

Analysis of capsaicinoids Capsaicinoids, including capsaicin and dihydrocapsaicin, were extracted from the fruit according to the procedure reported by Minami *et al.* (1998), with the following modifications. Ground freeze-dried fruit without peduncles (400 mg) was mixed in 20 mL of an extraction solvent consisting of equal volumes of acetone and ethyl acetate, and the mixture was shaken for 1 h at room temperature and centrifuged at 1292 × *g* and 4℃ for 10 min. The solvent extraction was repeated twice, and the volume of the supernatant was adjusted to 50 mL using the solvent. Capsaicinoids in the solution were analysed using a HPLC system with fluorescence detection. A Develosil ODS-SR-3 column (150 mm × 3 mm i.d., Nomura Chemical, Aichi, Japan) was maintained at 40℃ in a Shimadzu CTO-20 AC oven. A Shimadzu LC-20AB pump was operated in isocratic mode with a mobile phase containing equal volumes of 1% acetic acid aqueous solution and acetonitrile at a flow rate of 0.4 mL/min. The injection volume of samples and standards was 5 µL. The respective capsaicinoid peaks were monitored at 485 nm (excitation) and 530 nm (emission) with a Shimadzu RF-20Axs fluorescence detector, and the concentrations were calibrated by plotting peak area against standard concentrations and expressed as mg/100 g-FW. All assays were performed in triplicate.

Conversion to Scoville heat units The Scoville heat unit (SHU) is an index of chili pepper pungency and is related to the concentration of capsaicinoids, including capsaicin, dihydrocapsaicin and nordihydrocapsaicin, in the fruit (Gonzales-Zamora *et al*., 2013), and was calculated according to Equation 1:

$$SHU = [(CAP + DHC) \times 16.1 + nDHC \times 9.3] \quad \cdots\cdots \text{Eq. 1}$$

where *CAP* is the concentration (ppm) of capsaicin, *DHC* is the concentration (ppm) of dihydrocapsaicin and *nDHC* is the concentration (ppm) of nordihydrocapsaicin.

Analysis of volatile compounds Whole fruits without peduncles were frozen using liquid nitrogen and ground in a mortar. The ground sample (1 g) in the vial was stored in a freezer (−20℃) until analysis. Following the methods reported by Eggink *et al.* (2012), the stored sample was incubated at 30℃ for 10 min, and 100 mM EDTA-NaOH (1 mL, pH adjusted to 7.5 with NaOH) solution and ethyl nonanoate (40 µL, 0.11 mg/mL in ethanol) as an internal standard were added. Calcium chloride (2 g) was added to halt the enzymatic reaction and the vial was sonicated for 5 min. The solution (1 mL) was pipetted to a 10 mL crimp cap vial (Agilent Technologies, Santa Clara, CA, USA) and used for the SPME fiber exposure according to the methods reported by Junior *et al.* (2012). The volatile compounds were desorbed from the fiber in the GC injector (split ratio 1:1) at 250℃ for 1 min. The fiber was reconditioned after each analysis for 5 min. A GC 6890N (Agilent Technologies) equipped with a DB-wax (60 m × 0.25 mm × 0.25 µm) column was used for the quantification. The injector and flame ionization detector (FID) temperatures were set to 250℃. Initially, the oven was set to 40℃, held for 2 min, then ramped to 200℃ (2℃/min, 38 min). The resulting peaks were calibrated by FID response of the internal standard, and the content of aroma compounds was determined according to their respective peak areas. GC-MS analyses were performed on a GC 7890N (Agilent Technologies) coupled to a 5975C inert XL Mass Selective Detector and the separations were performed with a DB-wax (60 m × 0.25 mm × 0.25 µm) column using similar conditions as described for GC-FID. The injector and transfer line temperatures were set at 250℃, the carrier gas (helium) flow rate was 32 cm/s, the detector was operated in EI mode (70 eV, mass range = m/z 29 − 450), and the temperature for electron ion source and the interface were set at 230℃. The compounds were identified by matching the mass spectra fragmentation pattern with the NIST 2008 library, and by comparing the linear retention indices (RIs) of *n*-alkanes (C7-C28) with the literature. The identities were further confirmed by co-elution using reference standards.

Analysis of Oxygen Radical Absorption Capacity (ORAC) The antioxidant activity of the fruit sample was evaluated in terms of the ORAC value according to the method of Huang *et al.* (2002), with modifications. Briefly, ground freeze-dried fruit without peduncles (100 mg) was added to 1 mL of 75% methanol, and the mixture was sonicated for 5 min and centrifuged at 1631× *g* for 5 min. Methanol extraction was repeated twice, and the supernatant was assayed for ORAC. A diluted supernatant (25 µL) and 90 nM fluorescein solution (150 µL) were transferred to a black 96-well microplate. The microplate was immediately placed in a fluorescence microplate reader (Synergy™ HT, BioTek, Winooski, VT, USA) and agitated, and then left to stand at 37℃ for 7 min. Next, 25 µL of 160 mM AAPH as the peroxyl radical generator was immediately added to the well. The reaction temperature was maintained at 37℃, and fluorescence was monitored kinetically, with data taken every minute for 90 min, with fluorescent filters set at 485 nm (excitation) and 530 nm (emission). A Trolox curve was plotted and used as an external standard and the area under the curve (AUC) of relative fluorescence value was calculated according to Equation 2:

$$AUC = 0.5 + f_1/f_0 + ... + ... + f_{89}/f_0 + 0.5\ (f_{90}/f_0) \quad \cdots\cdots \text{Eq. 2}$$

where f_0 is the initial relative fluorescence reading at 0 min and f_i is the relative fluorescence reading at time *i*. The ORAC value was calculated using a quadratic regression equation relating the Trolox or sample concentration and AUC, and was expressed as

Table 1. Physical properties of Takanotsume and Shimatogarashi peppers

Chili pepper	Length (cm)	Diameter (cm)	Whole weight (g)	Seeds count	Seed weight (g)	Flesh weight (g)
Takanotsume	3.8 ± 1.0	0.6 ± 0.1	0.87 ± 0.43	26 ± 15	0.16 ± 0.11	0.70 ± 0.32
Shimatogarashi	2.3 ± 0.5	0.8 ± 0.1	0.63 ± 0.29	22 ± 12	0.19 ± 0.10	0.43 ± 0.18

Data are expressed as means ± standard deviation (n = 30)

Table 2. Color analysis of Takanotsume and Shimatogarashi peppers

Chili pepper	*L**	*a**	*b**	Δ*Eab*	*C*	*h*°
Takanotsume	41.92 ± 1.97	38.30 ± 2.58	30.75 ± 3.50	11.18	49.13 ± 4.09	38.66 ± 1.72
Shimatogarashi	48.71 ± 2.11	41.29 ± 1.60	39.12 ± 2.76		56.89 ± 2.87	43.40 ± 1.41

Data are expressed as means ± standard deviation (n = 6)

Table 3. Organic acid and capsaicinoid contents of Takanotsume and Shimatogarashi peppers

Chili pepper	Organic acids (mg/100 g-FW)			Capsicinoids (mg/100 g-FW)		Scoville heat unit
	Malic acid	Citric acid	L-Ascorbic acid	Capsaicin	Dihydrocapsaicin	
Takanotsume	51.1 ± 4.5	694.8 ± 62.9*	105.6 ± 2.7*	133.4 ± 7.1	153.9 ± 10.4	11900 ± 720
Shimatogarashi	613.0 ± 46.7*	154.8 ± 7.4	65.8 ± 1.1	289.5 ± 14.8*	238.7 ± 12.1*	29730 ± 1510*

Data are expressed as means ± standard deviation (n = 3). Asterisk (*) denotes significant difference analyzed by student *t*-test at $p < 0.05$.

μmol of Trolox equivalents (TE) per 100 g-FW. All assays were performed in triplicate.

Analysis of total phenolic content The sample for evaluation of total phenolic content was prepared as for the ORAC assay above. The total phenolic content of the sample was evaluated using the Folin-Ciocalteu method of Singleton and Rossi (1965), with slight modifications. Briefly, various concentrations of diluted sample (20 μL), distilled water (60 μL) and Folin-Ciocalteu reagent (15 μL, previously diluted 2-fold with distilled water) were transferred to a 96-well microplate (Nunc, Roskilde, Denmark) and mixed well. The microplate was immediately placed in a microplate reader (PowerWave™ XS2, BioTek) and agitated, and then allowed to stand for 15 min until stable absorption values were obtained. The absorbance was then measured at 750 nm. The total phenolic content was calculated from a linear gallic acid calibration curve and expressed as mg of gallic acid equivalents (GAE)/g-FW. All assays were performed in triplicate.

Statistical analysis The values of physical properties (n = 30), color analysis (n = 6) and the other measurements (n = 3) were expressed as means ± standard deviation. The statistical difference was determined using a two-sided Student's *t*-test. Differences were considered significant at $p < 0.05$ or 0.01.

Results and Discussion

Physical properties Shimatogarashi peppers were smaller and lighter than Takanotsume peppers (Table 1), and the physical properties were within the ranges reported by Jarret *et al.* (2007). The flesh to seed weight ratio was also lower in Shimatogarashi, which suggested that it had thinner flesh and less placental tissue compared to Takanotsume.

The color of mature fruits of each species was assessed. L^*, a^* and b^* values differed between species, and an 11.18 difference in magnitude (Δ*Eab*) (Table 2) was observed. The chroma and tone (hue angle) were also different. Shimatogarashi showed slightly higher lightness (L^*) compared to Takanotsume. The a^* and b^* values revealed that Shimatogarashi is lighter, and that the yellow and red colors were more saturated compared to Takanotsume. Compared to red Habanero (*C. chinense*) cultivars, the a^* and b^* values of both chilies were within the ranges reported by Pino *et al.* (2007). The red color was mainly due to the composition of capsanthin and capsorubin, the distinctive pigments in the genus *Capsicum* (Giuffrida *et al.,* 2013).

Organic acids The predominant organic acids in Shimatogarashi and Takanotsume were malic acid and citric acid, respectively (Table 3). Luning *et al.* (1994) reported malic, citric and ascorbic acids as the prevalent organic acids in fresh bell peppers (*C. annuum*). They also reported that total citric acid content, and *L*-ascorbic acid content to a lesser extent, contributed to sourness, while malic acid contributed negatively to sourness. Furthermore, Eggink *et al.* (2012) observed that among the organic acids in chilies, the citric acid content correlated well with sourness; however, the relationship between organic acids and sourness perception might not be significant, owing to possible interference from volatile and non-volatile compounds or texture differences of the fruit. The citric acid content in Shimatogarashi (154.8 ± 7.4 mg/g FW) was within the range for *C. annuum* L. (155 − 393 mg/g FW) reported by Matsufuji *et al.* (2007). The *L*-ascorbic acid contents (Table 3) reported for Shimatogarashi and

Takanotsume (65.8 and 105.6 mg/100 g FW, respectively) were slightly higher than the range reported by Topuz and Ozdemir (2007) for *C. annuum* L. (15.2 − 64.9 mg/100 g FW) using similar extraction methods.

Pungency Shimatogarashi had a nearly three-fold higher capsaicin and dihydrocapsaicin content than Takanotsume. The capsaicinoid content reported for Shimatogarashi and Takanotsume was within the range observed in previous reports for *C. frutescens* (Jarret *et al*., 2007) and *C. annuum* (Minami *et al*., 1994). As major compounds, capsaicin and dihydrocapsaicin comprised about 79 − 90% of the total capsaicinoid content during maturation (Barbero *et al*., 2014). Interestingly, the capsaicin to dihydrocapsaicin ratios in Shimatogarashi and Takanotsume were 1.15 and 0.82, respectively. Chili pepper pungency is expressed as SHU, and the calculated values are shown in Table 3. SHU is related to the concentrations of capsaicinoids, including capsaicin, dihydrocapsaicin and nordihydrocapsaicin, in chili (Gonzales-Zamora *et al*., 2013). Since other capsaicinoids, including nordihydrocapsaicin, in Takanotsume and Shimatogarashi were detected in trace quantities (data not shown), the SHU was also assessed using the concentration of capsaicin and dihydrocapsaicin alone in this study. Based on the SHU classification of pungency levels by Weiss (2002), Shimatogarashi can be classified as highly pungent and Takanotsume as moderately pungent. Another study by Al-Othman (2011) categorized five varieties of *C. annuum* L. as follows: hot chilies as highly pungent, red chilies as moderately pungent, green chilies as mildly pungent, and green, yellow and red bell peppers as non-pungent.

Volatile compounds Shimatogarashi and Takanotsume had 48 volatile compounds in common. 2-Hexenal, which has a strong impact on the aroma attributes of sweet peppers (*C. annuum*) according to Eggink *et al*. (2012), was prevalent in Shimatogarashi, while it was the second most common volatile compound in Takanotsume (Table 4). In Shimatogarashi, the peak area of hexanal was larger than in Takanotsume, which may suggest that Shimatogarashi had fresher notes compared to Takanotsume. Hexanal, 2-hexenal and hexanol were also reported by Ziino *et al*. (2009) as major compounds in Calabrian hot peppers (*C. annuum* L.).

Mazida *et al.* (2005) characterized hexanal and 2-hexenal as typical aroma compounds in chilies, in addition to linalool, 2,3-butanedione, 2-isobutyl-3-methoxypyrazine and 3-carene. In this study, 2,3-butanedione, 2-isobutyl-3-methoxypyrazine and 3-carene were not detected in either Shimatogarashi or Takanotsume peppers. However, we found 2-penten-1-ol in both chilies, which was described by Eggink *et al*. (2012) as a significant contributor to pepper flavor, imparting fruity/apple and sweetness attributes.

Aldehydes were prevalent volatile compounds in Shimatogarashi; while in Takanotsume, terpenoids were the major constituents (Fig. 1), with 10*s*,11*s*-himachala- 3(12),4-diene in highest concentration. The existence of this compound evidently distinguished the volatile profile of Takanotsume from Shimatogarashi. In contrast, Rodriguez-Burruezo *et al.* (2010) reported a relatively small amount of this volatile compound in one *C. frutescens* and one *C. annuum* studied. Kollmannsberger *et al.* (2011) noted that himachalenes are typical compounds found in more pungent *C. frutescens* and *C. annuum* varieties. However, α-himachalene was only detected in Takanotsume peppers.

Octadecanal, heptanol, *p*-cymene, hexyl 2-methyl propanoate, *(Z)*-3-hexenyl 2-methylbutanoate and pentanoic acid were found in Shimatogarashi but not in Takanotsume. Both hexyl 2-methylpropanoate and *(Z)*-3-hexenyl 2-methylbutanoate were described as having a strong fruity odor (Burdock, 2010). Bauer *et al*. (2001) mentioned that dehydrogenation of limonene led to the formation of *p*-cymene; thus, the absence of *p*-cymene in the volatile compound profile of Takanotsume might be related to the low level of limonene. On the other hand, *p*-menth-1-en-9-al, β-ocimene, ylangene, (*Z*)-β-farnesene, (*E*)-nerolidol and butanoic acid were found in Takanotsume, but not in Shimatogarashi (Table 4). Thus, *p*-menth-1-en-9-al (fruity notes) and β-ocimene (sweet, warm and herbaceous notes) might be key compounds in differentiating between the flavor characteristics of Shimatogarashi and Takanotsume peppers. Eggink *et al.* (2012) also indicated that *p*-menth-1-en-9-al and β-ocimene were important metabolites in determining flavor differences between genotypes and harvests of sweet pepper (*C. annuum*).

The ester content in Shimatogarashi was higher than in Takanotsume (Fig. 1). Many esters contribute to fruity flavors (Bauer *et al*., 2001). Namely, 3-methylbutyl 2-methylbutanoate, hexyl 2-methylpropanoate, *(Z)*-3-hexenyl 2-methylbutanoate, hexyl 2-methylbutanoate, hexyl 3-methylbutanoate and ethyl hexanoate were associated with fruity notes (Rodriguez-Burruezo *et al*., 2010; Burdock, 2010). While 3-methylbutyl 2-methylbutanoate, hexyl 2-methylbutanoate, hexyl 3-methylbutanoate and ethyl hexanoate were found in both chilies, hexyl 2-methylpropanoate and *(Z)*-3-hexenyl 2-methylbutanoate were only found in Shimatogarashi. Shimatogarashi had higher hexyl 2-methylbutanoate and hexyl-3-methylbutanoate contents than Takanotsume. These compounds are attributed to the fruity and sweet notes (Kollsmanberger *et al*., 2011).

Methyl salicylate was also found to be a major component in the volatile compound profile of both chilies, and was more prevalent in Shimatogarashi than in Takanotsume. This supports the results of Rodriguez-Burruezo *et al.* (2010), who found that methyl salicylate was detected in *C. frutescens* through smell, but was not noticeable in *C. annuum* and *C. chinense* varieties. Conversely, guaiacol, a trace compound found in capsaicin's thermal degradation products (Henderson and Henderson, 1992), was found in a larger concentration in Takanotsume than in Shimatogarashi. Among other phenolic volatiles, benzyl alcohol was found in small amounts in both chilies.

Table 4. Characterization of the volatile compounds in Takanotsume (as control) and Shimatogarashi peppers

RI[a]	Compound	TAK	SHI	Identification	Aroma description
	Aldehydes				
1078	hexanal	43.18 ± 0.28	48.53 ± 14.19	RI, MS, STD	grassy,[b] grass, tallow[c] fruity odor characteristic[d]
1124	2-pentenal	6.81 ± 0.46	16.03 ± 4.44*	RI, MS, STD	tomato, green, apple, orange, pungent[d], strawberry[e]
1213	2-hexenal	715.80 ± 115.29	853.23 ± 252.97*	RI, MS, STD	green, leaf,[c] fruity, almond, spicy, sweet[e]
1514	benzaldehyde	4.21 ± 0.51	4.60 ± 0.74	RI, MS, STD	almond, burnt sugar,[c] bitter almond[f]
1607	*p*-menth-1-en-9-al	8.62 ± 0.21	nd	RI, MS	spicy, herbal[g]
1976	pentadecanal	19.28 ± 5.79	23.66 ± 3.77*	RI, MS	fresh[c]
2080	octadecanal	nd	29.61 ± 5.09	RI, MS	-
	Alcohols				
1143	butanol	3.64 ± 2.35	2.27 ± 0.62	RI, MS, STD	medicinal, fruit[c], fusel-like, sweet and pleasant[d]
1159	1-penten-3-ol	4.11 ± 0.34	6.18 ± 1.20	RI, MS, STD	mild, grassy-green[d]
1250	pentanol	1.14 ± 0.11	2.66 ± 0.72	RI, MS, STD	balsamic[c], fusel-like, sweet and pleasant[d]
1315	4-methyl-1-pentanol	7.21 ± 3.93	34.72 ± 7.79*	RI, MS, STD	-
1320	2-penten-1-ol	6.18 ± 2.75	8.38 ± 1.00	RI, MS	plastic, rubber[c], green-diffusive[d]
1354	hexanol	2.18 ± 0.45	13.40 ± 2.34*	RI, MS, STD	resin, flower, green[c], fruity, bell pepper, herbal[e]
1384	*(Z)*-3-hexen-1-ol	1.58 ± 0.45	6.64 ± 1.82	RI, MS, STD	grass,[c] herbal, leafy[d]
1457	heptanol	nd	2.06 ± 0.67	RI, MS, STD	mushroom[c], woody, heavy, oily, fatty[d]
1491	2-ethyl-1-hexanol	15.88 ± 2.10	15.09 ± 5.36*	RI, MS, STD	oily, sweet, slightly floral, rose-like[d]
1875	benzyl alcohol	6.12 ± 1.55	4.08 ± 0.67	RI, MS, STD	flower,[c] pleasant, fruity,[d] sweet[f]
1962	2,6-dimethyl-3,7-octadiene-2,6-diol	7.57 ± 10.84	22.61 ± 4.54	MS	-
1972	dodecanol	6.63 ± 4.88	14.78 ± 4.69*	RI, MS, STD	fatty, waxy[d]
2178	tetradecanol	2.33 ± 0.47	2.09 ± 0.60	RI, MS, STD	coconut[c]
	Terpenoids				
1194	limonene	4.24 ± 1.47	6.37 ± 1.45	RI, MS, STD	citrus, mint,[c] lemon-like[f]
1241	γ-terpinene	1.14 ± 0.09	1.62 ± 0.50	RI, MS, STD	gasoline, turpentine,[c] herbaceous, citrus[f]
1247	*β*-ocimene	9.72 ± 5.34	nd	RI, MS	sweet, herb, [c]warm, herbaceous,[d] rancid[e]
1265	*p*-cymene	nd	1.09 ± 0.04	RI, MS, STD	solvent, gasoline, citrus,[c] lemon-like[d]
1275	terpinolene	8.26 ± 1.24	5.08 ± 3.26	RI, MS, STD	pleasant, sweet-piney[d]
1477	ylangene	7.38 ± 1.19	nd	RI, MS	-
1548	linalool	9.98 ± 7.92	6.49 ± 2.87	RI, MS, STD	citrus, fruity, floral,[b] lavender[c]
1599	1-terpinen-4-ol	1.24 ± 0.16	1.23 ± 0.24	RI, MS, STD	turpentine, nutmeg, must,[c] herbaceous[f]
1635	α-himachalene	17.39 ± 0.53	nd	MS	-
1663	*(Z)*-β-farnesene	5.87 ± 0.05	nd	RI, MS	citrus, green[c], fruity, herbaceous[d]
1704	α-terpineol	18.19 ± 6.14	8.78 ± 0.91	RI, MS, STD	oil, anise, mint[c],peach-like[d], lilac[f]
1719	10s,11s-himachala-3(12),4-diene	967.21 ± 56.30	nd	MS	-
1850	geraniol	3.66 ± 1.04	1.92 ± 0.92	RI, MS, STD	rose, geranium,[c] citrus[f]
2000	*(E)*-nerolidol	3.22 ± 1.43	nd	RI, MS, STD	wood, flower, wax[c], fresh, rose and apple-like[d]
	Esters				
1278	3-methylbutyl 2-methylbutanoate	1.87 ± 0.32	2.62 ± 0.55	RI, MS	fruity[d]
1340	hexyl 2-methylpropanoate	nd	9.32 ± 0.64	RI, MS	fruity[d]
1425	hexyl 2-methylbutanoate	1.06 ± 0.03	47.81 ± 40.78*	RI, MS	fruity,[b] sweet, exotic[h]
1443	hexyl 3-methylbutanoate	1.50 ± 0.06	15.09 ± 12.75*	RI, MS	fruity,[b] sweet, exotic[h]
1470	*(Z)*-3-hexenyl 2-methylbutanoate	nd	34.86 ± 35.39	RI, MS, STD	herb, sweet,[c] unripe apple, pineapple-like[d]
1952	ethyl hexanoate	4.70 ± 1.19	2.13 ± 0.48	RI, MS, STD	powerful, fruity, pineapple-banana note[d]

Table 4. continued

RI[a]	Compound	TAK	SHI	Identification	Aroma description
	Acids				
1446	acetic acid	1.10 ± 0.09	tr	RI, MS, STD	sour,[c] pungent, cider vinegar-like[d]
1585	2,2-dimethylpropanoic acid	66.15 ± 19.42	59.48 ± 9.10	RI, MS, STD	-
1625	butanoic acid	1.96 ± 0.12	nd	RI, MS, STD	rancid, butter-like[d]
1668	isovaleric acid	6.36 ± 1.88	2.04 ± 0.37	RI, MS, STD	sweat, acid, rancid,[c] cheese-like[d]
1687	3,3-dimethylbutanoic acid	18.84 ± 2.34	11.81 ± 5.29	RI, MS, STD	-
1737	pentanoic acid	nd	tr	RI, MS, STD	sweat,[c] unpleasant[d]
1805	4-methylpentanoic acid	nd	6.38 ± 1.19	RI, MS	unpleasant, sour[d]
1846	hexanoic acid	5.04 ± 2.87	1.81 ± 0.37	RI, MS, STD	sweaty, rancid, sour, sharp, pungent, cheesy[d]
1967	2-hexenoic acid	39.15 ± 46.33	22.61 ± 4.54	RI, MS, STD	must, fat,[c] green, earthy, sweet, fruity odor[d]
2056	octanoic acid	8.68 ± 6.35	10.16 ± 0.89	RI, MS, STD	sweat, cheese,[c] mildly unpleasant, fruity-acid[d]
2170	nonanoic acid	1.92 ± 0.47	nd	RI, MS, STD	green, fat,[c] cheesy, waxy[d]
2277	*n*-decanoic acid	2.26 ± 0.19	nd	RI, MS, STD	rancid, fat,[c] unpleasant[d]
	Alkanes				
1453	2-methyltetradecane	12.48 ± 3.91	16.05 ± 9.60	RI, MS	-
1499	pentadecane	4.93 ± 1.78	7.17 ± 2.03*	RI, MS, STD	alkane[c]
1598	hexadecane	1.24 ± 0.13	1.93 ± 1.00	RI, MS, STD	alkane[c]
1697	heptadecane	22.53 ± 16.18	16.09 ± 4.86	RI, MS, STD	alkane[c]
	Phenol derivatives				
1768	methyl salicylate	49.45 ± 18.27	164.60 ± 78.82*	RI, MS, STD	green, sweet, phenolic,[b] peppermint[c]
1856	guaiacol	51.88 ± 62.82	5.89 ± 0.56	RI, MS, STD	smoke, sweet, medicinal,[c] phenolic, hot[j]
	Norcarotenoids				
1850	α-ionone	1.78 ± 1.00	18.86 ± 16.61*	RI, MS, STD	floral,[b] wood, violet,[c] sweet[f]
1936	β-ionone	125.33 ± 18.44*	5.89 ± 0.56	RI, MS, STD	fruity, floral,[b] seaweed, raspberry,[c] cedarwood[f]

Data expressed as mean values ± standard deviation GC peak areas (n = 3)
Asterisk (*) indicated significantly higher amount between the two cultivars analyzed by student *t*-test at $p < 0.05$
TAK (Takanotsume); SHI (Shimatogarashi); tr (traces, content less than 1); nd (not detectable); MS (mass spectrum was in agreement with NIST 08 library); RI (retention index was in agreement with literature data); and STD (retention index was in agreement with standard co-elution).
[a]Retention Index on DB-Wax column (60 m × 0.25 mm × 0.25 µm) with homologous series of *n*-alkanes ($C_7 - C_{23}$); [b]Rodriguez-Burruezo *et al.,* (2010); [c]www.flavornet.org (i); [d]Burdock, (2010); [e]Luning *et al.,* (1994); [f]Bauer *et al.,* (2001); [g]the goodscentcompany.com (ii); [i]Kollsmannberger *et al.,* (2011); and [j]Shahidi and Naczk, (2003).

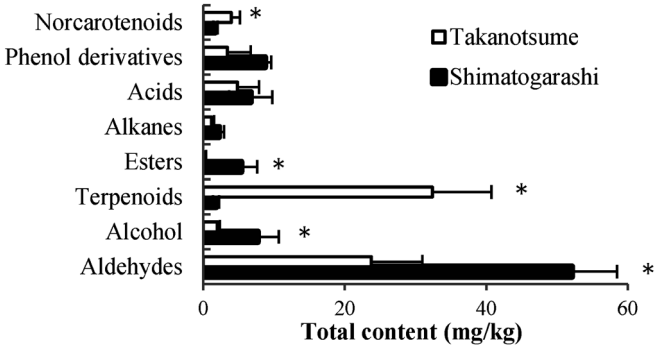

Fig. 1. Volatile compound classes in Takanotsume and Shimatogarashi peppers (n=3). Asterisk (*) denotes significant difference analyzed by student *t*-test at $p < 0.05$

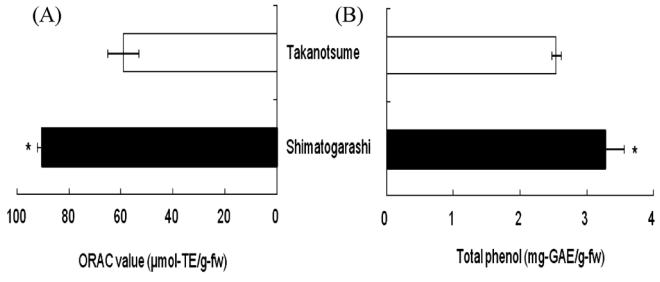

Fig. 2. Antioxidant properties of Takanotsume and Shimatogarashi peppers. (A) ORAC value and (B) total phenolic content. Data are expressed as means ± standard deviation (n=3). Asterisk (*) denotes significant difference analyzed by student *t*-test at $p < 0.05$.

The volatile compounds contributing to the floral-fruity aroma of *C. frutescens* and *C. annuum* (Rodriguez-Burruezo *et al*., 2010), i.e., α-ionone and β-ionone, were classified as norcarotenoids (Table 4). α-Ionone was more prevalent in Shimatogarashi, while β-ionone was more prevalent in Takanotsume.

Antioxidant properties The ORAC value of Shimatogarashi was significantly higher than that of Takanotsume. This might correlate with the total phenolic content in Shimatogarashi (3.28 ± 0.28 mg GAE/g FW), which was higher than in Takanotsume (2.54 ± 0.07 mg GAE/g FW). A positive correlation was also reported by Meckelmann *et al.* (2013) in *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens* varieties. The capsaicinoid content (Table 3) might also influence the phenolic content and ORAC due to their antioxidant properties (Li-E *et al*., 2008). The phenol derivatives in the volatile compound profile of Shimatogarashi also had a larger peak area compared to Takanotsume (Fig. 1). However, *L*-ascorbic acid did not appear to be correlated with the ORAC value, which is in agreement with the study of Alvares-Parrilla *et al.* (2011). Since phenolic compounds and the capsaicinoid content are important factors affecting the antioxidant activity of chilies, this information might lead to practical product development and quality control of chili products. Overall, the ORAC and total phenolic content for Shimatogarashi (90.65 ± 1.55 μmol TE/g FW and 3.28 ± 0.28 mg GAE/g FW) and Takanotsume (59.17 ± 6.06 μmol TE/g FW and 2.54 ± 0.07 mg GAE/g FW) shown in Fig. 2, were higher than for *C. annuum* (ORAC value of 12.67 μmol TE/g FW and total phenolic content of 1.70 mg GAE/g FW for red mature fruits) reported by Isabelle *et al*. (2010).

Conclusion

The physical properties, flavor quality attributes and antioxidant capacity were determined for Shimatogarashi in comparison to Takanotsume as a control. Physically, Shimatogarashi was smaller and brighter in color than Takanotsume. The Shimatogarashi pepper had a different organic acid content and was more pungent than Takanotsume, owing to the higher concentration of capsaicinoids. Shimatogarashi was characterized by a fresh, fruity aroma, attributed to its aldehyde and ester contents, while Takanotsume was warm and herbaceous, owing to its terpenoid content. Shimatogarashi exhibited higher antioxidant capacity compared to Takanotsume, attributable to its higher phenolic and capsaicinoid contents. The current results indicated that Shimatogarashi demonstrated unique characteristics in terms of physical, flavor and functional properties compared to the control cultivar. The flavor attributes and functional properties determined in this study can be utilized to differentiate between cultivars and their places of origin, and to confirm their authenticity.

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