Evaluating the Taste of Tomato Cultivated Under Salt-stress Conditions by Component Change, Sensory Evaluation, and Taste Sensor

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Tomatoes (Solanum lycopersicum L.) cultivated under salt-stress conditions offer enhanced contents of sugars, organic acids, and amino acids, and have a favorable taste. In this study, 3 tomato cultivars and the components of sugars (glucose, fructose, and sucrose), organic acids (citric acid and malic acid), and 17 amino acids of fruits cultivated under salt-stress (100 mM NaCl) conditions were investigated using HPLC systems. Taste was evaluated using a taste sensor and sensory evaluation. The taste sensor measured sourness, saltiness, bitterness, and astringency. In the sensory evaluation, the 5 basic tastes (sweetness, sourness, saltiness, umami, and bitterness), aroma, and texture were evaluated. Salt-stressed tomatoes undergo characteristic component changes due to the salt-stress, resulting in enhanced contents of glucose, fructose, citric acid, and several amino acids. The tendency toward increased saltiness in salt-stressed tomatoes was found by both the sensory evaluation and the taste sensor. Sourness was evaluated more sensitively and accurately by the taste sensor. To evaluate the objective tastes of a tomato, it is important not only to incorporate sensory evaluation but also to measure metabolic components using HPLC systems and to evaluate tastes using a taste sensor.

Keywords : amino acids, HPLC systems, NaCl, saltiness, sourness
using ingredient analysis, taste sensor analysis and sensory evaluation, and compared the distribution on the taste map (Wajima et al., 2012). When the correlation of the distribution with sensory evaluation was analyzed, the taste sensor analysis was higher than ingredient analysis, and the possibility of taste evaluation by taste sensor was suggested.

However, there have been no reports regarding to the changes of tastes of salt-stressed tomatoes by using taste sensors. In this study, to evaluate the taste of salt-stressed tomatoes, we investigated the components of sugars, organic acids, and amino acids using HPLC, evaluated taste using a taste sensor and sensory evaluation, and examined a taste assessment method that comprehensively considers these results.

MATERIALS AND METHODS

**Plant materials and growth conditions**

In our study, we used 3 cultivars of tomatoes (*Solanum lycopersicum* L.), where ‘Momotaro-8’ (Takii Seed Co., Japan) and ‘Reika’ (Sakata Seed Co., Japan) are known as large tomatoes, and ‘Frutica’ (Takii Seed Co.) as a medium-sized. On February 26, 2016, seeds were sown in vermiculite in a growth chamber at 28°C for 12 hours of light and 25°C for 12 hours of dark. Seedlings were transplanted into 12-cm pots filled with commercial soil (Tanemaki Baido; Takii Seed Co.) on March 28, 2016. On April 12, the plants were transplanted to a closed irrigation system of a culture bed (length×width×depth=5.1 m×30 cm×25 cm) filled with pumices (< 2 mm) in a greenhouse of the Prefectural University of Kumamoto. Ten plants per cultivar were transplanted into the culture bed. The experimental design consisted of 2 replications. The nutrient solution was OAT A at 1/4 strength (OAT Agrio Co., Japan).

**Salt treatment**

After 7 days of transplantation of the seedlings into the closed irrigation system, salt treatment was started by irrigation with OAT A nutrient solution supplemented with NaCl. For the acclimation of the plants to NaCl treatment, the concentration of salt was gradually increased by a 25 mM NaCl increment daily to 100 mM of final concentration. As a control, only OAT A nutrient solution was applied. The nutrient solution in each treatment was OAT A at 1/4 strength (OAT Agrio Co., Japan).

**Fruit harvest**

Red ripe fruits were harvested in the second and fourth trusses, and the weight and color of 9 or 10 fruits of each cultivar or treatment were recorded. Fruit color was measured in triplicate at the equator of each fruit using a colorimeter (Minolta CR-300, Minolta Co., Japan) in CIELAB units. Total soluble solids (TSS) were measured using a refractometer (PAL-1, Atago Co., Japan) for 6 samples. The fruits were stored at -30°C until analysis.

**Preparation of sample**

After the fruits were thawed, 100 g of each fruit was homogenized for 1 minute using a food processor and homogenized with ultrapure water of equal volume for 1 minute. The homogenate was centrifuged at 3,000 rpm for 10 minutes. The supernatant was used as a sample for measurement using an HPLC system and a taste sensor.

**Sugar, organic acid, and amino acid contents**

Each sample was filtered through a 0.45 μm filter (DISMIC®13CP045AN, ADVANTEC® Toyo Co., Japan). The filtrates were measured by an HPLC system using the method described by Zushi and Matsuzoe (2006). Measurement of the sugar contents (sucrose, fructose, and glucose) were quantified and separated using a Shim-pack SCR101C column (300×7.9 mm, Shimadzu Co., Japan) maintained at 80°C. Each sample was injected at 5 μL. The elution was performed with water at a flow rate of 0.8 mL min⁻¹. Sugar concentrations were monitored using a refractive index detector (RID-10A; Shimadzu Co.).

Measurement of the organic acid contents (malic acid and citric acid) were quantified and determined by a post-column-derivatization HPLC system. The separation was carried out using a Shim-pack SCR-102H column (300×8.0 mm, Shimadzu Co.) maintained at 40°C. The elution was performed with 5 mM p-toluenesulfonic acid at a flow rate of 0.8 mL min⁻¹. The post-column derivatization was carried out with 5 mM p-toluenesulfonic acid containing 20 mM Bis-Tris and 100 μM EDTA at a flow rate of 0.8 mL min⁻¹. The sample was injected at 10 μL. The organic acid concentrations were monitored using a conductivity detector (CDD-10A; Shimadzu Co.).

Amino acid contents were determined by the pre-column derivatization method using o-phthalaldehyde and 9-fluorenylmethyl chloroformate. Separation was carried out using an Inertsil ODS-4HP column (100×3.0 mm, GL Sciences Inc., Japan) maintained at 33°C, and the sample was injected at 1 μL. The elution was performed using the gradient by increasing solvent B (acetonitrile: methanol: water=45: 40: 15 volume) to solvent A (phosphate buffer in which was dissolved 17 mM potassium dihydrogen phosphate and 3 mM dipotassium hydrogen phosphate, pH 6.2) at the flow rate of 0.9 mL min⁻¹. The gradient used the following proportions: A/B (%)=90/10 at 0 minute, 72/28 at 6 minutes, 10/90 at 15 minutes, 0/100 at 16 minutes, 90/10 at 20.5 minutes. Amino acid concentrations were monitored at 350 nm (excitation) and 450 nm (emission) using a fluorescence detector (RF-20A; Shimadzu Co.).

**Taste measurement using taste sensor**

Taste was measured for sourness, saltiness, bitterness, and astringency using a taste sensor (TS-5000Z, Intelligent Sensor Technology Inc., Japan) at the Kumamoto Industrial Research Institute. The reference solution was a mixture of 30 mM KCl and 0.3 mM tartaric acid, equivalent to human saliva. The electric potential $Vr$ was measured in the reference solution, and the electric potential $Vs$ was measured in the sample. Then the bitterness sensor and astringency sensor were washed lightly, and electric potential $Vr$ was measured by re-immersing the sample in the reference solution. Each electric potential was measured in triplicate. Sourness, saltiness, bitterness (nigami-zatsumi), and astringency (shibumi-shigeki) of the initial taste were
calculated on the basis of the electric potential difference ($V_s - V_r$). The bitterness and astringency of the aftertaste were defined as the electric potential difference ($V_r' - V_r$).

**Sensory evaluation**

Sensory evaluation was carried out with 31 female students (around 20 years old) of the Prefectural University of Kumamoto in the university’s sensory laboratory from July 14–19, 2016. Sensory evaluation was performed between 2 pm and 4 pm so that the students could avoid eating and drinking one hour before the evaluation. Fruits were harvested on the day of evaluation. They were cut into half sections at the equator, then ‘Reika’ and ‘Momotaro-8’ were cut into eighth sections and ‘Frutica’ was cut into quarter sections. Panelists rinsed their mouths with distilled water before tasting each tomato section, and sections from each cultivar and treatment were presented to each panelist twice in random order. The strengths of the aroma, of the basic tastes (sweetness, sourness, saltiness, bitterness, and umami), and of the aftertaste were evaluated using a 5-point scale, as were the firmness of the flesh and of the skin, overall texture, and flavor.

**Statistical analysis**

The unpaired $t$-test, Kruskal–Wallis test, Mann-Whitney U-test, and one-way ANOVA following the Tukey-Kramer test were analyzed using JMP software version 9.0 (SAS Institute Inc., USA). Density estimation of 5-values of taste in sensory evaluation was created based on evaluation scores of 1 to 5 in 60–62 samples using R software version 3.5.2.

**RESULTS AND DISCUSSION**

**Changes in tomato fruits under the salt-stress treatment**

In tomatoes grown under the salt-stress treatment, water potential, water flow into the fruits, and fruit expansion are all reduced. Therefore, the fruit size and weight are also reduced (Cuartero and Fernández-Muñoz, 1999). Similar results were obtained in the present study. ‘Momotaro 8’ and ‘Reika’ fruits grown under the salt-stress treatment weighed 51% less than the control (Fig. 1A). ‘Frutica’ fruits weighed 22% less under the salt-stress treatment, but the difference vs. control was not significant. The a/b rate indicated that the strength of the reddish color was no change by the salt-stress treatment, and was the lowest in ‘Frutica’ (Fig. 1B). TSS significantly increased in all the 3 cultivars under the salt-stress treatment (Fig. 1C). The contents of sugars, organic acids, and

| Table 1 Changes in sugar and organic acid contents on fresh-weight basis (mg g$^{-1}$ FW) of tomato fruit grown under salt-stress. |
|----------------|----------------|----------------|----------------|
| **Cultivar**   | **Treatment**  | **Sugar**      | **Organic acid** |
|                |                | Glucose        | Fructose       | Sucrose        | Citric acid | Malic acid |
| ‘Momotaro 8’   | Control        | 8.4 c$^7$      | 11.0 c         | 1.9 b          | 18.2 b      | 0.07 ab    |
|                | Salt-stress$^8$| 22.0 b         | 21.9 b         | 2.6 a          | 35.1 a      | 0.05 cd    |
| ‘Reika’        | Control        | 11.9 c         | 13.8 c         | 1.8 b          | 21.8 b      | 0.09 a     |
|                | Salt-stress$^8$| 22.5 b         | 22.7 b         | 2.6 a          | 33.5 a      | 0.03 d     |
| ‘Frutica’      | Control        | 21.1 b         | 22.8 b         | 2.0 b          | 20.1 b      | 0.06 bc    |
|                | Salt-stress$^8$| 33.8 a         | 30.0 a         | 2.6 a          | 34.6 a      | 0.05 cd    |

$^7$ Salt-stressed tomatoes cultivated under 100 mM NaCl conditions.

$^8$ Data show the means of 6–7 samples. For each sugar and organic acid, different letters indicate significant differences at $P<0.05$ according to the Tukey-Kramer test.
Table 2  Change in amino acid contents on fresh-weight basis (mg 100 g \(^{-1}\) FW) of tomato fruit grown under salt-stress.

| Amino acid | Taste \(^{z}\) | 'Momotaro 8' Control | Salt-stress \(^{y}\) | 'Reika' Control | Salt-stress | 'Frutica' Control | Salt-stress |
|------------|----------------|----------------------|---------------------|----------------|-------------|-------------------|-------------|
| Glutamic acid | So, Um | 141.8 b \(^{a}\) 258.9 a | 138.0 b 237.5 a | 153.4 b 231.5 a |
| Aspartic acid | So | 32.2 d 54.9 a | 40.6 cd 51.8 ab | 36.9 cd 45.9 bc |
| Asparagine | So | 23.2 c 47.4 a | 32.9 b 39.4 ab | 10.1 d 15.7 cd |
| Glutamine | Sw | 80.3 c 181.1 a | 126.1 b 151.4 ab | 39.8 d 56.4 cd |
| Serine | Sw | 5.0 bc 18.3 a | 8.5 b 14.8 a | 4.0 c 8.0 b |
| Alanine | Sw | 4.1 c 12.0 b | 7.7 c 17.3 a | 6.8 c 12.9 b |
| Threonine | Sw | 3.4 c 8.3 a | 5.4 b 7.7 a | 2.9 c 4.5 bc |
| Glycine | Sw | 1.8 b 2.8 a | 1.6 b 1.8 b | 1.8 b 1.8 b |
| Isoleucine | Bi | 1.0 c 2.5 a | 2.4 a 2.5 a | 1.3 bc 2.0 ab |
| Leucine | Bi | 1.5 b 2.4 a | 2.4 a 2.4 a | 1.8 b 2.3 a |
| Phenylalanine | Bi | 4.0 c 6.9 a | 6.0 ab 6.9 a | 5.3 bc 5.9 ab |
| Methionine | Bi | 0.9 bc 1.2 a | 1.1 ab 1.3 a | 0.6 cd 0.4 d |
| Arginine | Bi | 4.7 bc 6.3 a | 7.2 a 6.0 ab | 3.5 c 4.4 c |
| Histidine | Bi | 5.0 c 8.4 a | 5.0 c 6.5 b | 3.6 d 5.1 c |
| Lysine | Bi, Sw | 4.3 c 6.0 ab | 6.1 a 5.3 abc | 4.9 bc 5.2 abc |
| Tyrosine | No taste | 1.3 abc 1.8 ab | 2.1 a 1.6 ab | 1.2 bc 0.6 c |
| GABA | No taste | 16.9 c 83.4 a | 40.6 bc 77.2 a | 19.1 c 64.3 ab |

\(^{z}\) Tastes of amino acids are sweetness (Sw), sourness (So), umami (Um), and bitterness (Bi) based on reports (Fuke, 1994; Kawai et al., 2012).

\(^{y}\) Salt-stressed tomatoes cultivated under 100 mM NaCl conditions.

\(^{x}\) Data show the means of 6 samples. For each amino acid, different letters indicate significant differences at \(P < 0.05\) according to the Tukey-Kramer test.

Fig. 2  Changes in taste measured using the taste sensor in control (A) and salt-stress treatment (B) fruits. Data showns are the means of 6 - 7 samples. Error bar shows the standard error. Different letters indicate significant differences at \(P < 0.05\) according to the Tukey-Kramer test.
amino acids are enhanced by salt-stress treatment (Zushi and Matsuzoe, 2006; 2015). In our study, in fruits grown by salt-stress treatment, sugar contents (glucose, fructose, and sucrose) and citric acid content were significantly higher than in the controls in all the 3 cultivars (Table 1). Sugar contents were different in tomato cultivars grown by the salt-stress treatment vs. the same cultivars grown under control conditions (Zushi and Matsuzoe, 2015). In our study, glucose and fructose contents were the highest in ‘Frutica’, and this result agreed with the results of TSS (Table 1 and Fig. 1C). Sucrose content was similar in all the 3 cultivars, and malic acid content was low in each cultivar and treatment (Table 1). In all the 3 cultivars, glutamic acid, γ-aminobutyric acid (GABA), serine, alanine, and histidine were significantly increased by the salt-stress treatment compared with the controls.

**Taste measurement using taste sensor**

Several studies have used taste sensors to evaluate the tastes of various foods such as vegetables, juices, teas, alcoholic drinks, and oils objectively rather than by sensory evaluation (Ghasemi-Varnamkhasti et al., 2010). In our study, the tastes of the salt-stressed tomatoes were evaluated using a taste sensor, which measured sourness, saltiness, bitterness, and astringency. In the controls, sourness, bitterness, and astringency of the initial taste and astringency of the aftertaste changed more significantly in ‘Momotaro 8’ than in the other cultivars (Fig. 2A). In ‘Frutica’, bitterness of the aftertaste was significantly increased. In salt-stressed tomatoes, differences in taste from variety to variety were small, and only bitterness of the initial taste was significantly lower in ‘Frutica’ than in the other 2 cultivars (Fig. 2B).

For all the 3 cultivars, sourness and saltiness of the initial taste were significantly increased by salt-stress treatment (Fig. 3). Organic acid, asparagine, aspartic acid, and glutamic acid contents are involved in sourness (Carangal et al., 1954; Jones and Scott, 1983; Fuke, 1994; Fuke and Ueda, 1996; Kawai et al., 2012), and an increase in sourness is thought to be due to an increase in citric acid, glutamic acid, aspartic acid, and asparagine contents by the salt-stress treatment. Salt-stress increases the Na content in fruits (Zushi et al., 2009; Ali and Ismail, 2014; Zushi and Matsuzoe, 2015); therefore, it is considered that saltiness increases in salt-stressed tomatoes.

In ‘Momotaro 8’, salt-stress treatment significantly increased the bitterness and astringency of the initial taste and the bitterness of the aftertaste (Fig. 3A). Aftertaste bitterness in ‘Reika’ was significantly decreased by salt-stress treatment (Fig. 3C). Several amino acids taste bitter to humans, especially isoleucine, leucine, phenylalanine, and tryptophan; on the other hand, methionine, arginine, histidine, and lysine have weak or moderate bitterness (Fuke, 1994; Fuke and Ueda, 1996; Kawai et al., 2012). However, those amino acid contents were increased by salt-stress treatment, but no increase in either initial taste or aftertaste bitterness was detected by the taste sensor. This may be a difference between humans and sensors. In future study, it will be necessary to evaluate the taste of amino acids using a taste sensor.

**Relationship between sensory evaluation and taste components**

In the controls, ‘Momotaro 8’ and ‘Reika’ were found to be significantly more sour and bitter, and significantly less sweet, than ‘Frutica’ (Fig. 4A). The fruits grown by the salt-stress treatment showed a similar tendency (Fig. 4B). Humans feel sweetness strongly in the order of fructose, sucrose, and glucose (Maehashi, 2011). The results of the evaluation of sweetness were consistent with the sugar contents of glucose and fructose (‘Frutica’ > ‘Momotaro 8’ and ‘Reika’).

In both the control and treatment groups, ‘Frutica’
was the highest sweet and the least sour, salty, and bitter (Fig. 4). However, the contents of citric acid, glutamic acid, aspartic acid, and asparagine, which are very sour, were equivalent in all the 3 cultivars (Table 2), whereas in the sensory evaluation, ‘Frutica’ was the least sour (Fig. 4). In tomato, taste depends on the balance between sugar and organic acid (Grierson and Kader, 1986). Contents of glucose and fructose were higher in ‘Frutica’ than in ‘Momotaro 8’ or ‘Reika’, and ‘Frutica’ was found to be the sweetest; therefore, it is considered that the sourness of ‘Frutica’ was evaluated as weak. The total value of amino acid contents (isoleucine, leucine, phenylalanine, methionine, arginine, histidine, and lysine) that humans find bitter was the lowest in ‘Frutica’ in the control and salt-stress treatments (Table 2), consistent with results of the sensory evaluation.

In all the 3 cultivars, saltiness and umami were significantly increased by salt-stress treatment (Fig. 4). It has been reported that GABA tends to enhance saltiness among the 5 basic tastes in humans (Sasaki et al., 2010). In the present study, GABA was significantly increased by salt-stress treatment in all the 3 cultivars (Table 2), suggesting that all the 3 cultivars showed high degrees of saltiness. Glutamic acid is related to umami (Fuke, 1994; Fuke and Ueda, 1996; Kawai et al., 2012), and the content was increased by salt-stress treatment (Table 2). Further, sweetness and aftertaste in ‘Momotaro 8’, and ‘Frutica’ were significantly increased by salt-stress treatment (Fig. 5A and 5B). In ‘Frutica’, salt-stress treatment significantly increased sourness and bitterness; the flesh and skin of the fruit became hard, and the texture significantly decreased (Fig. 5C). It is known that the flesh and skin become hard in tomatoes cultivated under salt-stress treatment conditions (Cuartero and Fernández-Muñoz, 1999). It has been reported that aroma and texture (e.g., firmness of flesh and thickness of skin) affect the taste of a tomato (Tamaki et al., 2003; 2004). In further study, it is important to investigate changes in the aroma and texture of salt-stressed tomatoes and the influences of aroma and texture on taste.

**Relationship between sensory evaluation and taste sensor**

In the salt-stressed tomatoes, the increase in saltiness was consistent between the sensory evaluation and the taste sensor. Evaluation of bitterness in the sensory evaluation and taste sensor differed depending on the combination of cultivars and salt-stress treatments. In ‘Momotaro 8’, bitterness at initial taste was significantly decreased and bitterness at aftertaste was slightly increased by salt-stress treatment as evaluated by the taste sensor (Fig. 3A), whereas in sensory evaluation there was no change in bitterness between the treatment groups (Fig. 5A). In ‘Frutica’, the bitterness of the aftertaste was slightly decreased by the salt-stress treatment as evaluated by the taste sensor (Fig. 3C), but bitterness in the sensory evaluation was slightly increased (Fig. 5C). Bitterness as measured by the sensory evaluation had the lowest evaluation point of all of the tastes and tended to be the most difficult taste to perceive (Fig. 6). Therefore, bitterness is thought to be susceptible to other tastes, and likewise the taste of tomatoes is thought to be strongly influenced by sweetness, sourness, saltiness, and umami.

Furthermore, the components related to sourness (citric acid, glutamic acid, aspartic acid, and asparagine) increased equally in all the 3 cultivars as a result of the salt-stress treatment (Table 1 and Table 2). The taste sensor evaluation showed that sourness significantly increased in all cultivars (Fig. 3), whereas in the sensory evaluation, no
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Fig. 5 Changes in the taste of salt-stressed tomatoes in sensory evaluation of ‘Momotaro 8’ (A), ‘Reika’ (B), ‘Frutica’ (C). For data, see the Fig. 4 legend. Asterisks indicate significant differences in the control vs. salt-stress treatment at $P < 0.05$ according to the Mann-Whitney U-test.

significant increase in sourness was observed in ‘Momotaro 8’ or ‘Reika’ (Fig. 5A and 5B). However, the amounts of change in sourness by the salt-stress treatment were small for both sensory evaluation (0.2–0.4) and the taste sensor (0.7–1.0) in ‘Momotaro 8’ and ‘Reika’, and the amounts of change in ‘Frutica’ were high: 0.7 and 2.9 in ‘Frutica’. It is thought that this reflects differences to sensory scores. Sensors 10: 3411–3443.

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Fig. 6 Density estimation of 5-values of taste in sensory evaluation. The graph was created using evaluation scores of 60 - 62 samples, and each evaluation score is graded on a scale of 1 to 5.

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