J. Biol. Macromol., **20** (2), 33-39 (2020)

Article

Isolation of phenolic components from strawberry cultivar 'Tokun' and their inhibitory activities on recombinant human histidine decarboxylase

Yoko Nitta[1*], Miyuki Mori[1], Yuji Noguchi[2], Yuichi Uno[3], Misaki Ishibashi[3], Hiroshi Ueno[4], Hiroe Kikuzaki[5]

[1]Department of Nutritional Science, Faculty of Health and Welfare Science, Okayama Prefectural University, Japan

[2]Division of vegetable breeding, Institute of Vegetable and Floriculture Science, NARO, Japan

[3]Plant Resource Science, Graduate School of Agricultural Science, Kobe University, Japan

[4] Laboratory of Applied Microbiology & Biochemistry, Ryukoku University, Japan,

[5]Department of Food Science and Nutrition, Faculty of Human Life and Environment, Nara Women's University, Japan

Received January 6, 2020; Accepted February 4, 2020

Histamine, known as bioactive amine, is synthesized through histidine decarboxylase (HDC). HDC is the sole enzyme that catalyzes histamine production in the human body and thus HDC inhibitor is expected to control histamine-mediating biological activity by controlling histamine production. In the present study, active compound that inhibits HDC activity was sought from strawberry cultivar 'Tokun' that was the most effective cultivar among 11 different cultivar of strawberries. From active fraction of ethyl acetate soluble part, tiliroside, a flavonoid glucoside, was isolated as an HDC inhibitor with IC_{50} = 40.3 µM. Casuarictin, an ellagitannin, was detected from some active fractions and showed potent inhibition with IC_{50} = 3.3 µM. Judging from potency of inhibition and content of 'Tokun' extract, casuarictin rather than tiliroside contributed to HDC inhibition of 'Tokun' extract more effectively. Tiliroside, on the other hand, was thought to be a possible marker to predict effective strawberry cultivar for HDC inhibition.

Key words: Histamine; flavonoid glycoside; tiliroside; ellagitannin; casuarictin

Introduction

Histidine decarboxylase (HDC) catalyzes the formation of histamine, which is associated with allergic and other biologic reaction in the human body. Agents that inhibit HDC activity would be beneficial for controlling allergy, for example. However clinically available HDC inhibitors have not been found because of difficulty in handling HDC even at purified form. Recent progress for understanding catalysis mechanism of HDC from X-ray crystal structure [1], which requires

*Correspondence author: Yoko Nitta.
Phone: +81-866-94-2143
Fax: +81-866-94-2202
E-mail: nitta@fhw.oka-pu.ac.jp

uniform and stable samples for crystallization, became HDC more easily accessible for seeking inhibitors from natural source. Previous studies have shown that *Rosaceae* family includes many plants whose extracts inhibit HDC activity [2]. Meadowsweet belongs to *Rosaceae* and contributed to the identification of ellagitannins as a potent HDC inhibitor [3]. Strawberry is one of the most popular fruits in *Rosaceae* consumed worldwide. Our previous study showed the 50 v/v % EtOH extract of strawberry fruits significantly inhibited HDC activity and among the 11 cultivars collected locally in japan, 'Tokun' had the most highest inhibition of HDC activity [4]. In this study the isolation and identification of the active compounds from the strawberry cultivar 'Tokun' were performed.

Materials and Methods

'Tokun' fruit was obtained from Institute of Vegetable and Floriculture Science, NARO, Japan on April 2016 and 2017. Casuarictin was purchased from Nagara Science Ltd. (Gifu, Japan). Chemicals were of analytical reagent grade.

The 70% aqueous acetone extract of 'Tokun' fruit (5 kg) was partitioned with hexane, CH_2Cl_2, and EtOAc, to obtain the respective soluble parts. The EtOAc soluble part (5.4 g) was separated by Chromatorex ODS (Fuji-silysia Chemical Ltd, Aichi, Japan) to give eleven fractions. Fraction 10 (66.8 mg) was subjected to Sephadex LH-20 (GE Healthcare, England, UK) column chromatography (CC) using CH_3OH as an eluent to give compound **1** (21.5 mg) [5]. Fraction 7 (156 mg) was also subjected to Sephadex LH-20 CC using CH_3OH as an eluent to give compound **2** (22.6 mg) [6]. NMR spectra were obtained from Ultrashield 300 (300 MHz, Bruker Co. USA) . LC-MS/MS were performed using Acquity TQD (Waters Co. USA)

Compound **1** (tiliroside): ^{1}H-NMR(CD_3OD): δ_H 7.99 (2H, d, J=8.9Hz), 7.40 (1H, d, J=15.9Hz), 7.32 (2H, d, J=8.6Hz), 6.82 (2H, d, J=8.9Hz), 6.79 (2H, d, J=8.6Hz), 6.31 (1H, d, J=2.1Hz), 6.12 (1H, d, J=2.1Hz), 6.07(1H, d, J=15.9Hz), 5.24 (1H, d, J=7.5Hz), 4.29 (1H, dd, J=11.9, 2.2Hz), 4.18 (1H, dd, J=11.9, 6.4Hz), 3.50-3.40 (4H, m). ^{13}C-NMR (CD_3OD): δ_C 178.0, 167.4, 164.5, 161.5, 160.1, 159.8, 157.9, 157.0, 145.1, 133.8, 130.8, 129.8, 125.7, 121.3, 115.4, 114.6, 113.3, 104.2, 102.6, 98.6, 93.4, 76.6, 74.4, 74.3, 70.3, 62.9. LC-MS/MS (positive-ion mode) m/z 595 $[M+H]^+$, 309, 147; (negative-ion mode) m/z 593 $[M-H]^-$, 285.

Compound **2** (1-*O*-*trans*-cinnamoyl-β-D-glucose): ^{1}H-NMR(CD_3OD): δ_H 7.82 (1H, d, J=15.9Hz), 7.66-7.63 (2H, m), 7.45-7.43 (3H, m), 6.62 (1H, d, J=15.9Hz), 5.62 (1H, d, J=7.9Hz), 3.88 (1H, dd, J=11.9, 1.9Hz), 3.72 (1H, dd, J=11.9, 4.6Hz), 3.49-3.39 (4H, m). LC-MS/MS (negative-ion mode) m/z 355 $[M-H]^-$, 147.

A test sample was dissolved in EtOH. 50 v/v % EtOH 'Tokun' extract was obtained from approximately 250 mg lyophilized powder in 500 μL of 50 v/v% EtOH, as reported previously [4]. Vortexed mixture was centrifuged at 20,000 g for 10 min to isolate supernatants.

The amounts of tiliroside and casuarictin in the extract were estimated with an HPLC pump equipped with a SHIMADZU SPD-20A Photo Diode Aray Detector. The column for HPLC was a Mightysil RP-18GP (5 μm, 250 x 4.6mm, Kanto Chemical). Solvent A was 0.2 % (v/v) formic acid (FA) in water and solvent B was 0.2% (v/v) FA in acetonitrile. The linear gradient elution used was as follows: 10-15% B in A over 15 min; 15-20% B in A over 40 min; 20-90% B in A over 20 min; 90% B in A held for 5min. The flow rate was 0.6mL/min. The wavelength was 280 nm. Tiliroside and casuarictin were detected with retention time of 74.2 and 34.4 min, respectively.

For HDC inhibition test, an active form of recombinant human HDC, that is a C-terminal

truncated form, was prepared as described previously [3]. HDC inhibition assay mixture contained 0.1 mM dithiothreitol, 0.01 mM PLP, a test sample and enzyme in 100 mM potassium phosphate buffer (pH 6.8) and reaction was initiated by addition of L-histidine to be 0.8 mM at 37 °C. The final volume of the assay was 200 µl, including 10 µl strawberry sample so as to give a final concentration of 0.1% for the hexane and CH_2Cl_2 extracts and the EtOAc and H_2O-soluble fractions, or 0.15–150 µM for the isolated compound. After 20 min incubation the reaction was terminated by addition of 10 µl of 60% perchloric acid. The histamine produced in the assays was measured by injecting the aliquot of the assay mixture onto an HPLC system equipped with a histamine Pak column (Tosoh, Tokyo, Japan). Separated histamine was fluorometrically measured by using the *o*-phthalaldehyde method as described previously [3].

Results and Discussion

From the inhibition rate and yield of each soluble part, it was found that the EtOAc soluble part contributed about 80 % of the total inhibition of the 50 v/v % EtOH extract of 'Tokun' fruits. Further fractionation of the EtOAc soluble part by Chromatorex ODS gave eleven fractions. HPLC chromatogram showed the peaks around 80-100 min in all fractions, which came from the solvent. At 0.1% concentration, inhibition of fraction 1-3 on HDC activity was negligible (Table 1). Among the highly active fractions 4–11, which exhibited an inhibition higher than 90% (Table 1), fraction 10 showed a single peak in HPLC analysis (Fig. 1). Fraction 10 was purified by Sephadex LH-20 (Fig. 2) to produce one compound that was identified as a flavonoid glycoside, kaempferol 3-*O*-[6" –(*E*)-*p*-coumaroyl]-β-D- glucose (tiliroside, compound **1** (Fig.3)) from LC-MS/MS, ^{1}H-NMR, ^{13}C-NMR and reference data [5]. Tiliroside inhibited HDC activity (IC_{50} = 40.3±4.8 µM), showing the highest inhibition of HDC among flavonoids reported previously as showing HDC inhibition [7, 8].

Although contribution of the fraction 7 to HDC was not observed (Table 1), large and single peak appeared at 280 nm by HPLC analysis (Fig. 1) and thus it was further fractionated by Sephadex LH-20, from which compound **2** were isolated (Fig. 2). Compound **2** was identified as 1-*O*-*trans*-cinnamoyl-β-D-glucose from LC-MS/MS, ^{1}H-NMR and reference data [6]. This compound showed insignificant inhibition on HDC activity. We tried to isolate further active compounds, however the amount of the other compounds was not enough to identify the chemical structure.

In the previous studies, ellagitannins were isolated as HDC inhibitors from meadowsweet [3]. Ellagitannnins were reported to be contained in strawberry ripe fruits [9] where casuarictin was one of the main ellagitannins in strawberries. The peak deriving from casuarictin was detected at 280 nm with retention time of 34.4 min in Fr. 4, 5, 6 and 8 (Fig. 1). Excepting the peak with retention time of 52 min (compound **2**), other peaks were smaller than that of casuarictin (Fig. 1), suggesting that the content of casuarictin was probably highest among ellagitannins in EtOAc extract of 'Tokun'. Casuarictin showed potent inhibition of HDC activity with IC_{50} = 3.3 ± 0.1 µM.

The content of tiriloside and casuarictin in 50 v/v % EtOH extract were estimated as 10 ± 2.4 and 7.8 ± 1.8 µM, respectively. The contribution of tiriloside and casuarictin to HDC inhibition of 50 v/v% EtOH extract of 'Tokun' was estimated as ~

ly, from the content and inhibitory rate. As reported previously ellagitannins (tellimagrandin II, rugosin A and D) inhibited HDC potently and it is probable that ellagitannins including casuarictin in 'Tokun' inhibited more potently than other phenolic compounds. Many peaks appeared in HPLC

chromatogram of Fr. 8 and 9 at 280 nm, suggesting that other compounds, probably ellagitannins, contributed to HDC inhibition.

Although the contribution of tiliroside to HDC inhibition was lower than that of casuarictin, as shown in Fig. 4, there was a correlation of the tiliroside content in five strawberry cultivars with the inhibitory ratio of HDC activity. The tested five cultivars were grown under the same condition in a greenhouse [4]. This suggests that tiliroside might be a possible marker to predict effective strawberry cultivar for HDC inhibition. To verify this assumption, more various kinds of cultivars should be examined. This will be performed in future study.

Foods containing high amount of ellagitannins like pomegranate [10] and *Rubus* berries [11] have been traditionally consumed expecting health benefit. Strawberry is also ellagitannin-rich fruit and the present study suggests that functionality of strawberry might be correlated with the control of histamine synthesis by its ellagitannins. Further studies are necessary to clarify the effect of ellagitannin-rich foods on biological activity via inhibition of HDC activity and it will be performed in the future.

Conclusions

Tiliroside was isolated as a HDC inhibitor from strawberry cultivar 'Tokun'. Tiliroside showed the highest inhibition of HDC among flavonoids reported previously as showing HDC inhibition. However casuarictin showed potent inhibition on HDC and contribution of casuarictin was much higher than that of tiliroside to HDC inhibition of 50 v/v% EtOH extract of 'Tokun'. As observed in other plants belonging to *Rosaceae* family, it was suggested that ellagitannins in 'Tokun' inhibited HDC activity mainly. For the development of HDC inhibitors from natural products, ellagitannins might be promising compounds. Activities of ellagitannins *in vivo* would be investigated in future studies.

Table 1. The contribution of Fr. 1-11 of EtOAc soluble parts to HDC inhibition.

Fr.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Contribution rate(%)	0	0	0	2.4	19.2	15.6	0	27.5	25.1	8.4	1.8

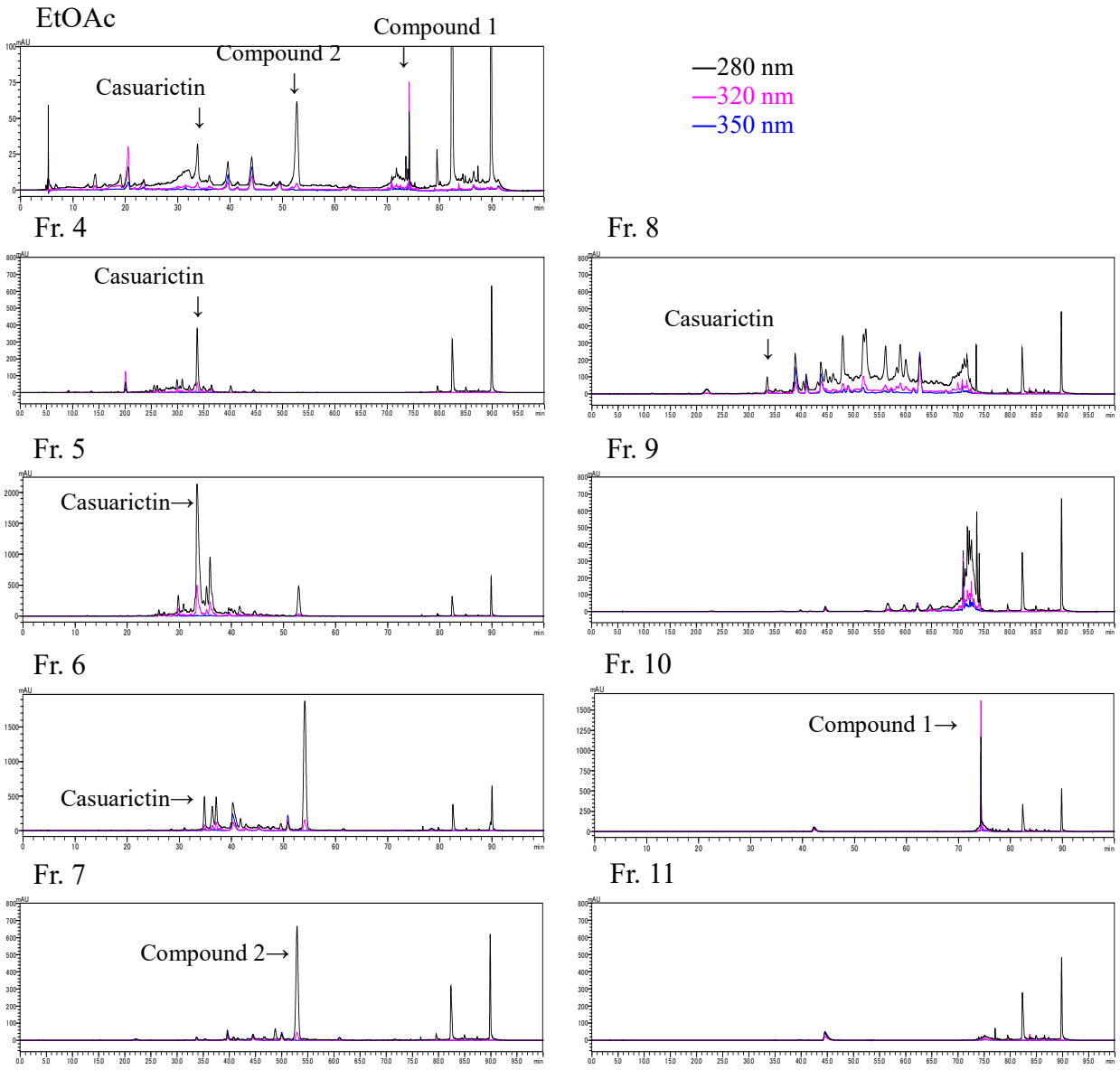

Fig. 1. HPLC chromatograms of EtOAc soluble parts and Fr. 4-11 of EtOAc soluble parts. RP-18 column, 30°C, 0.6 ml/min, 280 nm, 320nm, 350nm, A: 0.2% formic acid in H_2O, B: 0.2% formic acid in CH_3CN, B of A: 0-15 min, 10-15%; 15-55 min, 15-20%; 55-75 min, 20-90%; 75-80 min, 90%; 80-82 min, 90-10%; 82-100 min.

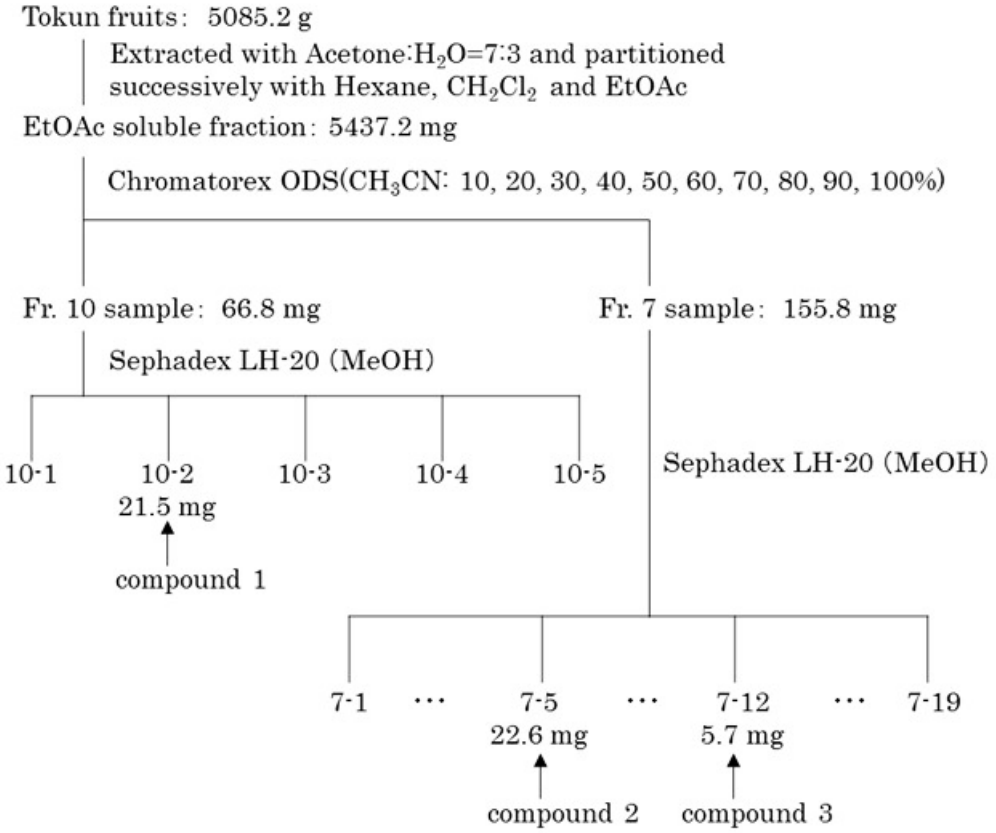

Fig. 2. Fractionation of 'Tokun' fruits and isolation of compound **1** and **2**.

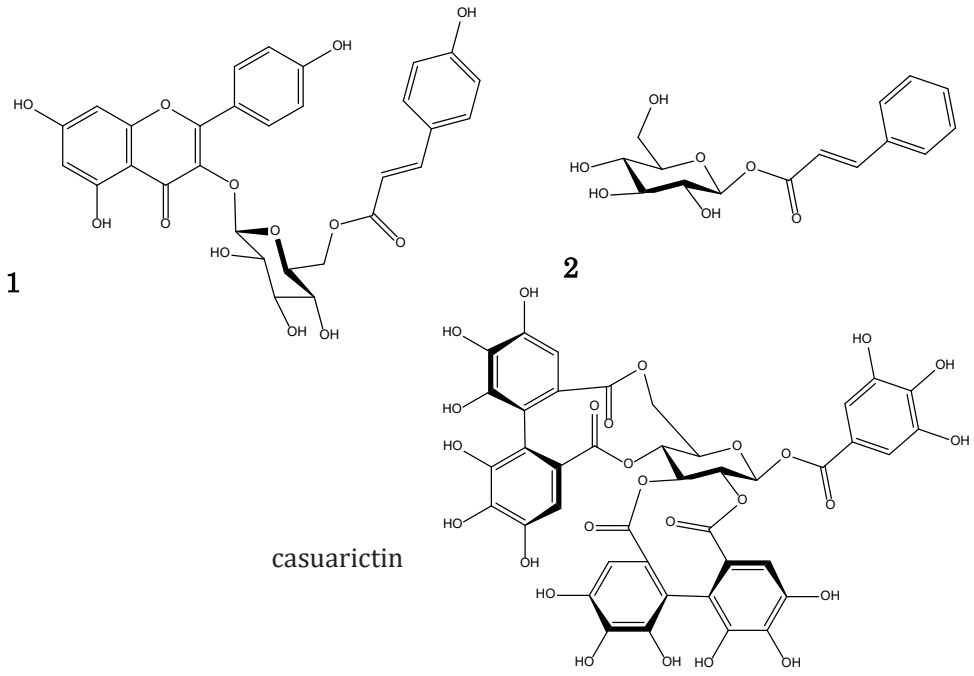

Fig. 3. Chemical structure of compound **1**and **2** and casuarictin.

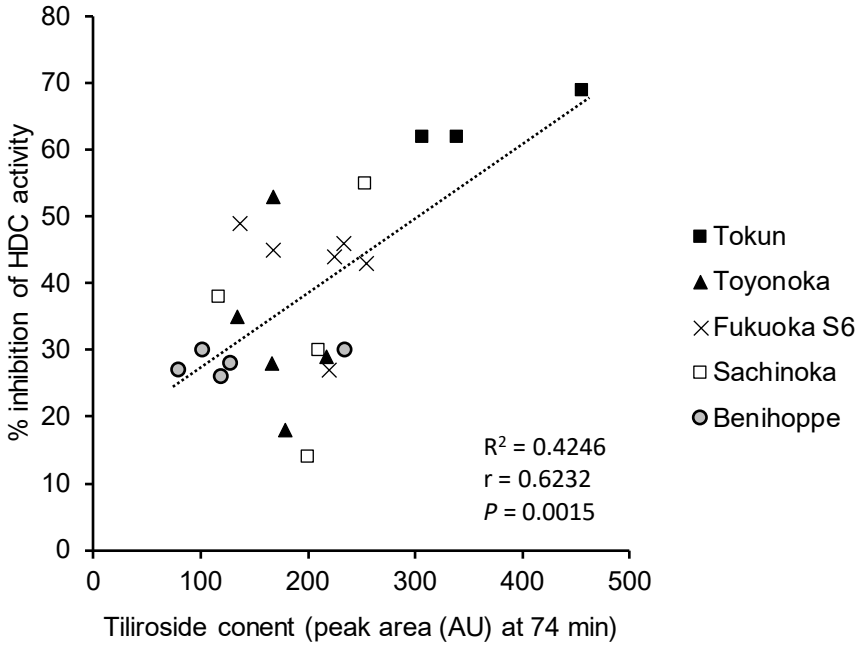

Fig. 4. Correlation of the tiliroside content in five strawberry cultivars with the inhibitory ratio of histidine decarboxylase (HDC) activity (%).

References

[1] Komori, H., Y. Nitta, H. Ueno, and Y. Higuchi. *J. Biol. Chem.*, (2012) **287**, 29175-29183.

[2] Nitta, Y., H. Kikuzaki, and H. Ueno. *Int. Biol. Rev.*, (2017) **1**, 1-14.

[3] Nitta, Y., H. Kikuzaki, T. Azuma, Y. Ye, M. Sakaue, Y. Higuchi, H. Komori, and H. Ueno. *Food Chem.*, (2013) **138**, 1551-1556.

[4] Uno, Y., Y. Nitta, M. Ishibashi, Y. Noguchi, and H. Kikuzaki. *Acta Physiologiae Plantarum*, (2017), 134-139.

[5] Zhang, Y., N.P. Seeram, R. Lee, L. Feng, and D. Heber. *J. Agric. Food Chem.*, (2008) **56**, 670-675.

[6] Ninomiya, M., T. Itoh, S. Ishikawa, M. Saiki, K. Narumiya, M. Yasuda, K. Koshikawa, Y. Nozawa, and M. Koketsu. *Bioorg. Med. Chem.*, (2010) **18**, 5932-5937.

[7] Nitta, Y., H. Kikuzaki, and H. Ueno. *J. Agric. Food Chem.*, (2007) **55**, 299-304.

[8] Nitta, Y., H. Kikuzaki, and H. Ueno. *Food Chem.*, (2009) **113**, 445-449.

[9] Gasperotti, M., D. Masuero, G. Guella, L. Palmieri, P. Martinatti, E. Pojer, F. Mattivi, and U. Vrhovsek. *J. Agric. Food Chem.*, (2013) **61**, 8597-8607.

[10] Ito, H., P. Li, M. Koreishi, A. Nagatomo, N. Nishida, and T. Yoshida. *Food Chem.*, (2014) **152**, 323-330.

[11] Sangiovanni, E., U. Vrhovsek, G. Rossoni, E. Colombo, C. Brunelli, L. Brembati, S. Trivulzio, M. Gasperotti, F. Mattivi, E. Bosisio, and M. Dell'Agli. *PLoS One*, (2013) **8**, e71762.

Communicated by Keiko Momma