

Assessment of Capsiconinoid Composition, Nonpungent Capsaicinoid Analogues, in *Capsicum* Cultivars

YOSHIYUKI TANAKA,^{*,†} MUNETAKA HOSOKAWA,[†] KEIGO OTSU,[†] TATSUO WATANABE,[‡]
 AND SUSUMU YAZAWA[†]

[†]Graduate School of Agriculture, Kyoto University, Oiwake-cho, Kitashirakawa, Kyoto 606-8502, Japan,
 and [‡]School of Food and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka
 422-8526, Japan

Capsiconinoid is a group of nonpungent capsaicinoid analogues produced in *Capsicum* fruits, which we recently identified. Capsiconinoids have agonist activity for transient receptor potential vanilloid type 1 (TRPV1), which is reported to be a receptor for capsaicin. It is, therefore, important to screen cultivars containing high levels of capsiconinoid for their use as a vegetable or dietary supplement. This study describes the quantitative analysis of capsiconinoid content in fruits of 35 *Capsicum* cultivars: 18 cultivars of *C. annuum*, 7 of *C. baccatum*, 5 of *C. chinense*, 4 of *C. frutescens*, and 1 of *C. pubescens*. Using high-performance liquid chromatography (HPLC), we found that 10 cultivars contained capsiconinoids. Capsiconinoid Baccatum (CCB) (*C. baccatum* var. *praetermissum*) showed the highest capsiconinoid content (3314 $\mu\text{g/g}$ DW) and Charapita (*C. chinense*) had the second highest content. The other 8 cultivars had much lower capsiconinoid content than these two cultivars (<300 $\mu\text{g/g}$ DW). Time-course analysis during fruit development clarified that capsiconinoid content in CCB fruits increased until 30 days after flowering (DAF) and then decreased rapidly until 40 DAF.

KEYWORDS: *Capsicum*; nonpungent capsaicinoid analogue; capsiconinoid; capsinoid; capsaicinoid

INTRODUCTION

The pungency of *Capsicum* fruits is caused by lipophilic alkaloid capsaicin and its analogues, called capsaicinoids (1). The fundamental chemical structure of capsaicinoids is an acid amide of vanillylamine with a fatty acid. Capsaicinoids are reported to have many bioactivities, for example, enhancement of thermogenesis and fat consumption in mammals (2, 3). These multiple physiological functions are due to the activation of the capsaicin receptor. The capsaicin receptor has been recently identified and termed transient receptor potential vanilloid type 1 (TRPV1) (4, 5).

We previously found a compound group from fruits of a nonpungent cultivar of *C. annuum*, CH-19 Sweet (6, 7), and named it capsinoid. The basic structure of capsinoids is an ester of vanillyl alcohol with a fatty acid. Compared to capsaicin, capsiate, a member of capsinoids, has similar TRPV1 agonist potential but considerably lower pungency (8). Several reports confirmed that capsiate promotes thermogenesis or endurance capacity through TRPV1 activation (9, 10). Moreover, various potencies of capsinoids such as suppression of fat accumulation, apoptosis induction, and anticancer and antioxidant properties were reported (11–16). Because of their low pungency, these capsaicinoid analogues are more palatable than capsaicinoids. From this perspective, nonpungent capsaicinoid analogues are attractive ingredients of dietary supplements.

Recently, we reported 2 nonpungent capsaicinoid analogues from the fruits of *C. baccatum* var. *praetermissum* (17). These compounds are coniferyl esters, named capsiconiate (coniferyl (*E*)-8-methyl-6-nonenoate) and dihydrocapsiconiate (coniferyl 8-methylnonanoate) (Figure 1). It was shown that capsiconiate and dihydrocapsiconiate have agonist activity for TRPV1 and that their pungency is very low, similar to that of capsinoids (17). We named this coniferyl ester group capsiconinoid. The physiological activities of capsiconinoids are being further investigated by our colleagues.

There are many studies on capsaicinoid content in various pepper cultivars or accessions (18–21). In a previous paper, we reported capsinoid content in various *Capsicum* cultivars (22). However, information on the capsiconinoid content in the fruits of *Capsicum* cultivars has not yet been published. The aim of this study was to determine the capsiconinoid content (capsiconiate and dihydrocapsiconiate) of various *Capsicum* cultivars by means of high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Plant Material. As shown in Table 1, 35 *Capsicum* cultivars were used for this experiment: 18 cultivars of *C. annuum*, 7 of *C. baccatum*, 5 of *C. chinense*, 4 of *C. frutescens*, and 1 of *C. pubescens*. All plants were grown at the experimental farm of Kyoto University (35.1° N, 135.5° E), from March to October, 2006.

Extraction of Capsiconinoid, Capsinoid, and Capsaicinoid. To determine capsiconinoid, capsinoid, and capsaicinoid content in each cultivar, 3–10 fruits at approximately 30 days after flowering (DAF)

*Corresponding author. Tel: +81-75-753-6048. Fax: +81-75-753-6068. E-mail: kapipara1014@yahoo.co.jp.

were used. The content was determined as mentioned in our previous report (23), with some modifications. After pericarps were cut with a knife, whole fruits were dried completely in a freeze drier (FDU-540, EYELA) for 3 days. Dried fruits were ground in a blender (MK-61M, National) at room temperature. A 4-mL volume of acetone (Wako) was added to

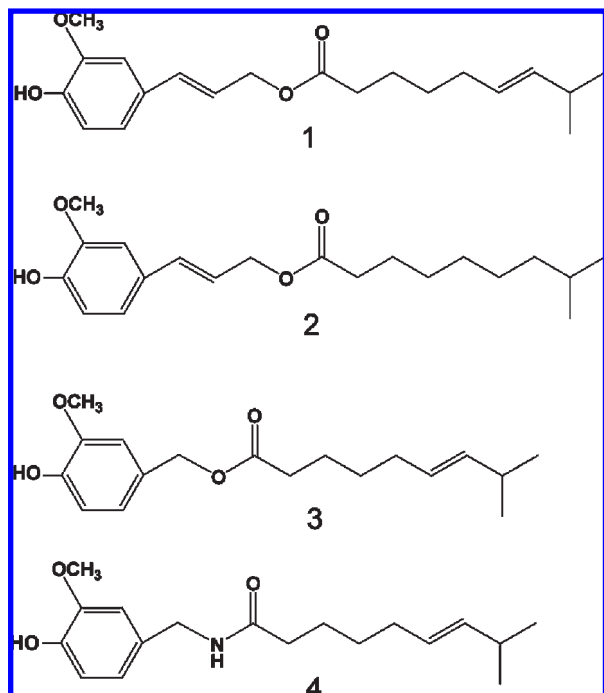


Figure 1. Chemical structures of capsiconiate (1), dihydrocapsiconiate (2), capsiate (3), and capsaicin (4).

0.2 mg of dry fruit powder. After vortexing, the sample tube was allowed to settle for 15 min at room temperature. The supernatant was collected, and 1 mL of acetone was added to the residue. The supernatant was collected again. After repeating this process, 1 mL of ethyl acetate was added to the residue, and the supernatant was collected. The combined supernatant volume was completely evaporated in a rotary evaporator (VC-960, Taitec) at 36 °C under vacuum. The residue was dissolved in 2 mL of ethyl acetate, and this solution was filtered into a 2-mL glass vial using Sep-pak Cartridge C18 (Waters) and then used for HPLC (Hitachi L-7420 system) analysis.

HPLC Analysis Conditions. The separation was performed on a μ -Bondapak C18 column (10 μ m, 3.9 mm \times 150 mm, Waters) coupled with a guard column (μ -Bondapak Guard-Pak, Waters). The eluent was a mixture of MeOH/H₂O (70:30 v/v) at a flow rate of 1.0 mL/min. Detection was performed at 280 nm with a UV detector. Injection volumes and run-time were 10 μ L and 30 min, respectively. The typical chromatograms of pepper fruits and standard compounds are shown in **Figure 2**.

Standards and Quantification. Standards of capsiconiate, capsiate, and capsaicin were used for retention time verification and quantification by HPLC. Standards of capsiconiate and capsiate were obtained by chemical synthesis (17, 24). Mixtures of capsaicinoid (Merck) were used as standards. A five concentration series, 10, 50, 100, 500, 1000 mg/L, was prepared for constructing a standard curve by dissolving capsiconiate in ethyl acetate. Each point of the curve was obtained from an average of 3 injections. Each substance was quantified by UV-vis peak area measurements. The standard curve was produced using a linear regression program (**Figure 3**). Total capsiconinoid content in fruits was calculated using the sum of capsiconiate and dihydrocapsiconiate contents. The standard curve of capsiconiate was also used to calculate dihydrocapsiconiate content. A standard curve of capsiate and capsaicin was prepared in the same manner as described above for capsiconiate. The total contents of capsinoid and capsaicinoid were calculated as the sum of contents of capsiate and dihydrocapsiate, and capsaicin and dihydrocapsaicin, respectively.

Change in Capsiconinoid Content during Fruit Development. Capsiconinoid Baccatum (CCB) was used for this analysis. The fruits of

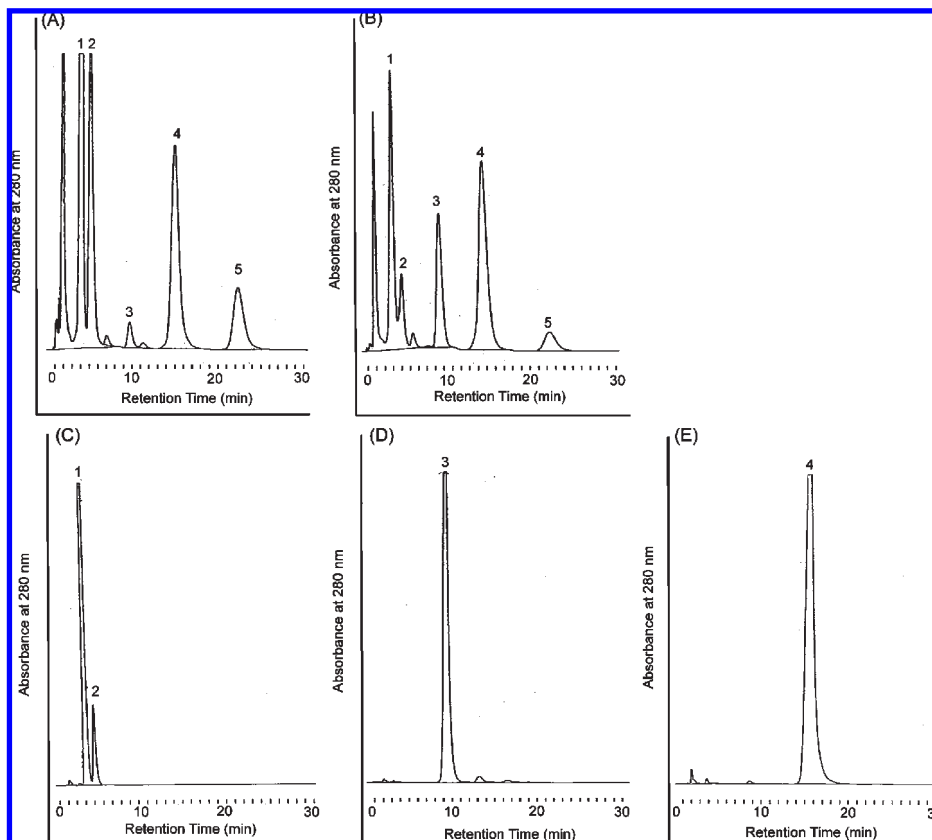


Figure 2. HPLC chromatograms of (A) CCB (*C. baccatum* var. *praetermissum*), (B) Charapita (*C. chinense*), (C) standard capsaicin (a mixture of capsaicin (85%) and dihydrocapsaicin (15%), Merck), (D) standard capsiate, and (E) standard capsiconiate. Capsaicin (1), dihydrocapsaicin (2), capsiate (3), capsiconiate (4), and dihydrocapsiconiate (5).

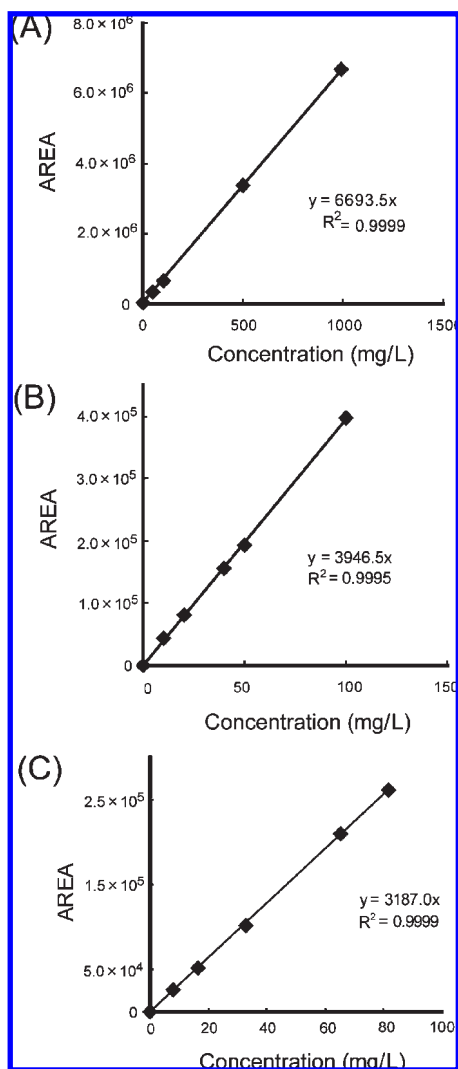


Figure 3. Standard curves for HPLC analysis. (A) Capsiconiate; (B) capsiate; (C) capsaisin.

CCB were collected in the fall of 2006. Flowers were tagged at anthesis in order to determine the stage of fruit growth, and randomly selected fruits were harvested at 15, 20, 30, 35, and 40 DAF. The capsiconinoid content (capsiconiate and dihydrocapsiconiate) in each fruit was determined by HPLC as described above. HPLC analysis was performed 3 times for each growth stage.

RESULTS AND DISCUSSION

Variation in Capsiconinoid Content in *Capsicum*. This study demonstrated the quantitative analysis by HPLC of capsiconinoid content in nonpungent capsiconinoid analogues. Total capsiconinoid content was evaluated using the sum of capsiconiate and dihydrocapsiconiate contents. Among 35 cultivars, 2 cultivars, Capsiconinoid Baccatum (CCB) (*C. baccatum* var. *praetermissum*) and Charapita (*C. chinense*) contained notably higher amounts of capsiconinoid than other cultivars (Table 1). The content of capsiconinoid in CCB and Charapita fruits was 3314 and 2694 $\mu\text{g/g}$ DW, respectively. Eight cultivars contained small amounts of capsiconinoid (< 300 $\mu\text{g/g}$ DW), and they were not detected in the other 25 cultivars. Only a small number of *Capsicum* cultivars contained capsiconinoids. However, capsiconinoids were detected in 4 cultivars of *C. annum*, 3 of *C. baccatum*, 2 of *C. chinense*, and 1 of *C. frutescens* at various concentrations. It was shown that capsiconinoid content is not species-specific but that capsiconinoids can exist ubiquitously in

Capsicum. Cultivars collected in South America, Africa, and Japan contained capsiconinoids (Table 1), which indicates that cultivars containing capsiconinoids are not geographically limited.

Determination of Capsiconiate and Dihydrocapsiconiate Content. Two capsiconinoid components, capsiconiate and dihydrocapsiconiate, were identified in *Capsicum* fruits. In this experiment, capsiconiate and dihydrocapsiconiate contents were determined by HPLC. In all cultivars containing capsiconinoids, the amount of capsiconiate was more than that of dihydrocapsiconiate (Table 2). It was reported that the major capsiconinoids in pepper fruits are capsaicin and dihydrocapsaicin, which are amides of vanillylamine with a medium chain (C = 10) branched fatty acid (25). The fatty acid portion of these capsiconinoids is the same as that of capsiconiate and dihydrocapsiconiate. The ratio of capsaicin to dihydrocapsaicin can vary substantially within and among species (26). Interestingly, the ratio of capsiconiate to dihydrocapsiconiate correlates with that of capsaicin to dihydrocapsaicin. Both ratios in Charapita are rather higher than those in other cultivars (Tables 2 and 3). For example, in Charapita, the ratio of capsiconiate to dihydrocapsiconiate was 10.2, and the ratio of capsaicin to dihydrocapsaicin was 5.1. However, the ratios in CCB were 3.6 and 1.8, respectively. This correlation indicates that capsiconinoids can be produced by condensation of coniferyl alcohol with a fatty acid, which is a common precursor in the capsiconinoid biosynthesis pathway. Capsiconinoids are produced by condensation of vanillylamine with a fatty acid (27). It is reported that capsiconinoid biosynthesis is controlled by the *Pun1* locus. *Pun1* encodes a putative acyltransferase, which conducts the acylation of vanillylamine with a fatty acid to form capsiconinoids (28). This putative acyltransferase gene, *Pun1*, might be involved in capsiconinoid biosynthesis.

Composition of Capsiconinoid, Capsinoid, and Capsaicinoid in *Capsicum* Cultivars. Compounds of 3 groups, capsiconinoid, capsinoid, and capsaicinoid, were identified from *Capsicum* fruits (1, 6, 7, 17). In this experiment, capsiconinoids and nonpungent capsiconinoid analogues were not detected in *C. annum* Fushimi-ama and 2 bell-type sweet pepper cultivars. On the whole, in pungent cultivars, capsiconinoids were the dominant compounds, and nonpungent capsiconinoid analogues were the minor compounds (Table 1). As an exception, CCB and Charapita contain much higher level of capsiconinoids, which is comparable to that of capsiconinoids. These results show that capsiconinoids were detected only in pungent cultivars and that there is considerable variation in capsiconinoid content among them. Moreover, variation in capsinoid content was confirmed in this study. CH-19 Sweet characteristically contained notably high amounts of capsinoid with very low amounts of capsiconinoid, which is consistent with our previous study (22). In addition, some pungent cultivars contained remarkably high amounts of capsinoid. For example, Charapita and Shima fruits contained 1800 and 2000 $\mu\text{g/g}$ DW capsinoids, respectively. These contents were higher than those of most pungent cultivars. It is interesting that capsiconinoids were found at a level comparable to those of both capsinoids and capsiconinoids in Charapita, which is unique among the 35 cultivars in this study (Table 1).

Nonpungent capsiconinoid analogues, capsiconinoids and capsinoids, can be biosynthesized through the same pathway as capsiconinoids. Sutoh et al. demonstrated that capsiconinoids were biosynthesized from phenylalanine and valine, common precursors for capsiconinoids (29). It was proposed that capsiconinoids are biosynthesized by condensation of a fatty acid and vanillyl alcohol, while capsiconinoids are biosynthesized by condensation of a fatty acid and vanillylamine. Capsiconinoids also have a remarkable structural resemblance to capsiconinoids and

Table 1. *Capsicum* Cultivars Used for This Study and Capsiconinoid, Capsinoid, and Capsaicinoid Content in Fruits^a

species	cultivar	country to collect	$\mu\text{g/g DW fruit}$		
			capsiconinoid	capsinoid	capsaicinoid
<i>C. annuum</i>	Ecadorian Hot Pepper	Ecuador	239.2 ± 26.6	807.3 ± 69.0	7442.7 ± 372.3
	CH-19 Sweet	Japan	75.5 ± 1.5	5632.5 ± 280.0	109.7 ± 3.1
	Bolivian rainbow	Bolivia	79.0 ± 0.2	228.2 ± 4.8	4741.1 ± 64.6
	Corn Red	Japan	11.8 ± 7.5	145.2 ± 19.3	6993.1 ± 625.2
	Patagonia	Chile	nd. ^b	1021.6 ± 164.3	6141.1 ± 540.8
	Corn Yellow	Japan	nd.	397.6 ± 47.4	7503.2 ± 183.2
	Yatsufusa	Japan	nd.	145.4 ± 27.8	3706.8 ± 993.5
	No. 3446	Peru	nd.	135.1 ± 25.5	5568.8 ± 368.5
	Nikko	Japan	nd.	50.7 ± 35.6	1054.2 ± 443.9
	Chameleon	Japan	nd.	22.3 ± 11.2	3291.2 ± 267.2
	Ordone	U.S.A.	nd.	nd.	4649.4 ± 376.3
	Hungarian Yellow Wax	U.S.A.	nd.	nd.	1353.9 ± 65.5
	Escondida	Chile	nd.	nd.	927.0 ± 12.3
	Chimayo	U.S.A.	nd.	nd.	201.1 ± 112.3
	Noumi-kurojishi	Japan	nd.	nd.	880.2 ± 232.0
	Fushimi-ama	Japan	nd.	nd.	nd.
	California Wonder	Japan	nd.	nd.	nd.
Orange Bell	Japan	nd.	nd.	nd.	
<i>C. baccatum</i> var. <i>praetermissum</i>	CCB	Mexico	3314.6 ± 357.4	45.7 ± 5.1	7885.3 ± 477.1
<i>C. baccatum</i> var. <i>baccatum</i>	Mexico1	Mexico	30.3 ± 1.6	264.8 ± 18.6	5250.2 ± 111.4
	No. 3188	Peru	nd.	506.4 ± 149.0	1950.1 ± 133.4
<i>C. baccatum</i> var. <i>pendulum</i>	Super	Côte d'Ivoire	106.7 ± 15.5	67.3 ± 1.0	5073.3 ± 93.5
	Peruvian orange	Peru	nd.	70.3 ± 6.9	905.6 ± 72.5
	Paraguay red	Paraguay	nd.	61.3 ± 12.8	1197.7 ± 47.3
	UFO	U.S.A.	nd.	nd.	511.0 ± 20.9
<i>C. chinense</i>	Charapita	Peru	2694.1 ± 91.9	1801.9 ± 23.8	5036.7 ± 200.6
	Sy-2	Seychelles	83.8 ± 7.9	1046.2 ± 112.1	7329.8 ± 325.4
	Peruvian Shiny Red	Peru	nd.	897.1 ± 136.5	9062.6 ± 618.3
	Habanero	Japan	nd.	nd.	7246.2 ± 30.5
	Guyana Tragedy	Guyana	nd.	nd.	2329.2 ± 224.0
<i>C. frutescens</i>	Costa Rica15	Costa Rica	39.3 ± 2.0	170.0 ± 12.3	7980.1 ± 413.4
	Shima	Japan	nd.	2056.6 ± 165.7	12148.4 ± 1000.0
	Tabasco	Japan	nd.	nd.	5012.9 ± 324.7
	Af-erect	Kenya	nd.	nd.	2451.0 ± 365.9
<i>C. pubescens</i>	Indonesia-1	Indonesia	nd.	nd.	3621.7 ± 541.1

^a Mean ± standard error ($n = 3$). ^b nd.: not detected.

Table 2. Capsiconiate and Dihydrocapsiconiate Content in *Capsicum* Fruits^a

species	cultivar	$\mu\text{g/g DW fruit}$		c/d ^c
		capsiconiate	dihydrocapsiconiate	
<i>C. annuum</i>	Ecadorian Hot Pepper	200.3 ± 23.1	38.8 ± 3.5	5.2
	CH-19 Sweet	48.4 ± 1.5	27.1 ± 0.2	1.8
	Bolivian Rainbow	59.9 ± 0.1	19.0 ± 0.3	3.1
	Corn Red	8.3 ± 5.2	3.5 ± 3.5	2.4
<i>C. baccatum</i> var. <i>praetermissum</i>	CCB	2594.6 ± 272.6	720.0 ± 84.9	3.6
<i>C. baccatum</i> var. <i>pendulum</i>	Super	69.3 ± 11.5	37.4 ± 4.3	1.9
<i>C. baccatum</i> var. <i>baccatum</i>	Mexico1	30.3 ± 1.6	nd. ^b	
<i>C. chinense</i>	Charapita	2467.0 ± 100.3	227.1 ± 8.4	10.9
	Sy-2	83.8 ± 7.9	nd.	
<i>C. frutescens</i>	Costa Rica15	39.3 ± 2.0	nd.	

^a Mean ± standard error ($n = 3$). ^b nd.: not detected. ^c Ratio of capsiconiate to dihydrocapsiconiate.

capsinoids, except for their coniferyl alcohol moiety. This indicates that capsiconinoids could be biosynthesized by condensation of a fatty acid and coniferyl alcohol through the same pathway as capsaicinoids and capsinoids. Cultivars containing large quantities of nonpungent capsaicinoid analogues, capsiconinoids or capsinoids, are required for use as a vegetable or dietary supplement. Understanding of the factors determining the composition of the 3 analogues is important to allow the breeding of cultivars containing large amounts of nonpungent capsaicinoid analogues; these factors are now under investigation. As described above, Charapita has a unique composition, containing

capsiconinoids at a level comparable to both capsaicinoids and capsinoids. This cultivar can be used for the investigation of the factors that determine the composition of capsaicinoids and its nonpungent analogues.

Change in Capsiconinoid Content in Developing Fruits. Change in capsiconinoid content in CCB plants was investigated by HPLC analysis. The capsiconinoid level increased progressively between 15 and 25 DAF, reaching 3800 $\mu\text{g/g DW}$ at 35 DAF (Figure 4). The capsaicinoid level also increased between 15 and 35 DAF, together with the capsiconinoid level. The capsiconinoid and capsaicinoid levels decreased after 35 DAF. The increase in

Table 3. Capsaicin and Dihydrocapsaicin Content in *Capsicum* Fruits^a

species	cultivar	$\mu\text{g/gDW}$ fruit		c/d ^c
		capsaicin	dihydrocapsaicin	
<i>C. annuum</i>	Ecuadorian Hot Pepper	4360.6 \pm 195.0	3082.1 \pm 177.4	1.4
	CH-19 Sweet	109.1 \pm 3.1	nd. ^b	
	Bolivian Rainbow	2674.6 \pm 73.6	2066.6 \pm 9.0	1.3
	Corn Red	4326.1 \pm 364.6	2667.0 \pm 3.5	1.6
<i>C. baccatum</i> var. <i>praetermissum</i>	CCB	5047.6 \pm 222.1	2837.8 \pm 192.2	1.8
<i>C. baccatum</i> var. <i>pendulum</i>	Super	2419.8 \pm 107.1	2653.5 \pm 39.1	0.9
<i>C. baccatum</i> var. <i>baccatum</i>	Mexico1	3093.1 \pm 137.4	2157.2 \pm 87.4	1.4
<i>C. chinense</i>	Charapita	4205.0 \pm 206.5	830.7 \pm 5.9	5.1
	Sy-2	4611.2 \pm 167.1	2718.6 \pm 221.0	1.7
<i>C. frutescens</i>	Costa Rica15	4973.8 \pm 220.2	3007.1 \pm 199.3	1.7

^a Mean \pm standard error ($n = 3$). ^b nd.: not detected. ^c Ratio of capsaicin to dihydrocapsaicin.

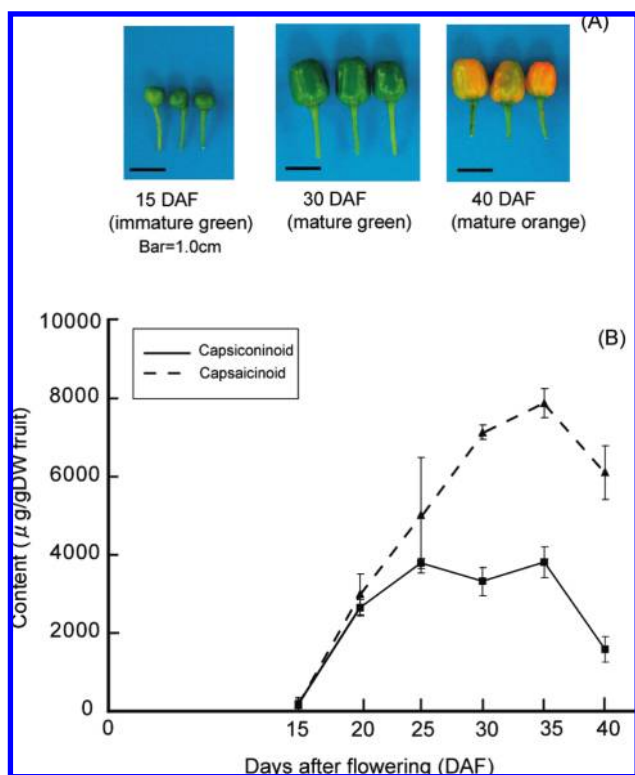


Figure 4. Change of capsinonoid and capsaicinoid content during CCB fruits development. (A) Fruit color and size change through fruit development and maturation. (B) Change of capsinonoid and capsaicinoid content during fruit development. Vertical bars indicate standard errors ($n = 3$).

capsinonoid level is similar to that in the capsaicinoid and capsinoid levels reported previously (22, 30, 31). Iwai et al. reported that in Karayatsubusa (*C. annuum*), capsaicinoids were first detected at 20 DAF and that accumulation increased gradually and reached a maximum level at 40 DAF (30). Then capsaicinoid concentration decreased significantly at 50 DAF. The capsinoid level in CH-19 Sweet (*C. annuum*) increased between 10 and 30 DAF and then decreased after 30 DAF (22). In order to obtain a high level of capsinonoids, mature green fruits should be collected at approximately 30 DAF, when capsinonoid content reaches its maximum level in CCB fruits (Figure 4). As CCB fruits mature, capsaicinoid content decreased by 22%. At 40 DAF when the fruits turned orange, capsinonoid content fell by a greater degree, i.e., 58%. These results indicate that capsinonoids can decompose more easily in fruits than capsaicinoids. As with the capsinonoid content in CCB,

capsinoid content decreased rapidly as CH-19 Sweet fruits matured (22). Capsinoids, which are esters of a fatty acid and vanillyl alcohol, are stable in nonpolar solvents such as ethyl acetate but decompose easily in polar solvents, such as water, methanol, and so forth (32). Capsaicinoids, which are amides of a fatty acid and vanillylamine, are stable in both polar and nonpolar solvents. It is estimated that capsinonoids, which are esters of a fatty acid and coniferyl alcohol, can decompose easily in polar solvents. This instability in water can be responsible for the rapid decrease of capsinoid and capsaicinoid contents in pepper fruits. It has also been reported that capsaicinoids are oxidized in fruits by peroxidases (31). Peroxidases also can be involved in the decrease of capsinoids and capsaicinoids.

In conclusion, this study showed that CCB and Charapita contain large amounts of capsinonoid, whereas the other cultivars contain little or no capsaicinoids ($< 300 \mu\text{g/g DW}$). The content of capsaicinoids in CCB and Charapita fruits was 3314 and 2694 $\mu\text{g/g DW}$, respectively. The capsinonoid content in CCB reached its maximum level in green mature fruits and then decreased rapidly as the fruits matured and turned orange. Capsinonate and dihydrocapsinonate have agonist activity for TRPV1 with low pungency; therefore, capsinonoids could be attractive food ingredients. When capsinonoids are used in food supplements, CCB and Charapita will be their important natural sources.

LITERATURE CITED

- (1) Nelson, E. K.; Dawson, L. E. The constitution of capsaicin, the pungent principle of Capsicum. III. *J. Am. Chem. Soc.* **1923**, *45*, 2179–2181.
- (2) Kawada, T.; Hagihara, K.; Iwai, K. Effects of capsaicin on lipid metabolism in rats fed a high fat diet. *J. Nutr.* **1986**, *116*, 1272–1278.
- (3) Kawada, T.; Sakabe, S.; Aoki, N.; Watanabe, T.; Higeta, K.; Iwai, K.; Sugimoto, E. Intake of sweeteners and pungent ingredients increases the thermogenin content in brown adipose tissue of rat. *J. Agric. Food Chem.* **1991**, *39*, 651–654.
- (4) Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **1997**, *389*, 816–824.
- (5) Szallasi, A. Vanilloid (capsaicin) receptors in health and disease. *Am. J. Clin. Pathol.* **2002**, *118*, 110–121.
- (6) Kobata, K.; Todo, T.; Yazawa, S.; Iwai, K.; Watanabe, T. Novel capsaicinoid-like substances, capsiate and dihydrocapsiate, from the fruits of a nonpungent cultivar, CH-19 Sweet, of pepper (*C. annuum* L.). *J. Agric. Food Chem.* **1998**, *46*, 1695–1697.
- (7) Kobata, K.; Sutoh, K.; Todo, T.; Yazawa, S.; Iwai, K.; Watanabe, T. Nordihydrocapsiate, a new capsinoid from the fruits of a nonpungent pepper *C. annuum*. *J. Nat. Prod.* **1999**, *62*, 335–336.
- (8) Iida, T.; Moriyama, T.; Kobata, K.; Morita, A.; Murayama, N.; Hashizume, S.; Fushiki, T.; Yazawa, S.; Watanabe, T.; Tominaga, M. TRPV1 activation and induction of nociceptive response by a

- non-pungent capsaicin-like compound, capsiate. *Neuropharmacol.* **2003**, *44*, 958–967.
- (9) Onuki, K.; Niwa, S.; Maeda, S.; Inoue, N.; Yazawa, S.; Fushiki, T. CH-19 Sweet, a non-pungent cultivar of red pepper, increased body temperature and oxygen consumption in humans. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 2033–2036.
- (10) Haramizu, S.; Mizunoya, W.; Masuda, Y.; Ohnuki, K.; Watanabe, T.; Yazawa, S.; Fushiki, T. Capsiate, a nonpungent capsaicin analog, increases endurance swimming capacity of mice by stimulation of vanilloid receptors. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 774–781.
- (11) Kawabata, F.; Inoue, N.; Yazawa, S.; Kawada, T.; Inoue, K.; Fushiki, T. Effects of CH-19 sweet, a non-pungent cultivar of red pepper, in decreasing the body weight and suppressing body fat accumulation by sympathetic nerve activation in humans. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 2824–2835.
- (12) Onuki, K.; Haramizu, S.; Oki, K.; Watanabe, T.; Yazawa, S.; Fushiki, T. Administration of capsiate, a non-pungent capsaicin analog, promotes energy metabolism and suppresses body fat accumulation in mice. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 2735–2740.
- (13) Macho, A.; Sancho, R.; Minassi, A.; Appendino, G.; Lawen, A.; Muñoz, E. Involvement of reactive oxygen species in capsaicinoid induced apoptosis in transformed cells. *Free Radical Res.* **2003**, *37*, 611–619.
- (14) Macho, A.; Lucena, C.; Sancho, R.; Daddario, N.; Minassi, A.; Muñoz, E.; Appendino, G. Non-pungent capsaicinoids from sweet pepper, synthesis and evaluation of the chemopreventive and anti-cancer potential. *Eur. J. Nutr.* **2003**, *42*, 2–9.
- (15) Masuda, Y.; Haramizu, S.; Oki, K.; Ohnuki, K.; Watanabe, T.; Yazawa, S.; Kawada, T.; Hashizume, S. Upregulation of uncoupling proteins by oral administration of capsiate, a nonpungent capsaicin analog. *J. Appl. Physiol.* **2003**, *95*, 2408–2415.
- (16) Rosa, A.; Deiana, M.; Casu, V.; Paccagnini, S.; Appendino, G.; Ballero, M.; Dessi, M. A. Antioxidant activity of capsinoids. *J. Agric. Food Chem.* **2002**, *50*, 7396–7401.
- (17) Kobata, K.; Tate, H.; Iwasaki, Y.; Tanaka, Y.; Ohtsu, K.; Yazawa, S.; Watanabe, T. Isolation of coniferyl esters from *Capsicum baccatum* L., and their enzymatic preparation and agonist activity for TRPV1. *Phytochemistry* **2008**, *69*, 1179–1184.
- (18) Topuz, A.; Ozdemir, F. Assessment of carotenoids, capsaicinoids and ascorbic acid composition of some selected pepper cultivars (*Capsicum annum* L.) grown in Turkey. *J. Food Compos. Anal.* **2007**, *20*, 596–602.
- (19) Cisneros-Pineda, O.; Torres-Tapia, L. W.; Gutiérrez-Pacheco, L. C.; Contreras-Martín, F.; González-Estrada, T.; Peraza-Sánchez, S. R. Capsaicinoids quantification in chili peppers cultivated in the state of Yucatan, Mexico. *Food Chem.* **2007**, *104*, 1755–1760.
- (20) Jarret, R. L.; Baldwin, E.; Perkins, B.; Bushuway, R.; Guthrie, K. Diversity of fruit quality characteristics in *Capsicum frutescens*. *HortScience* **2007**, *42*, 16–19.
- (21) Canto-Flick, A.; Balam-Uc, E.; Bello-Bello, J. J.; Lecona-Guzmán, C.; Solís-Marroquín, D.; Avilés-Viñas, S.; Gómez-Uc, E.; López-Puc, G.; Santana-Buzzy, N.; Iglesias-Andreu, L. G. Capsaicinoids content in Habanero pepper (*Capsicum chinense* Jacq.): Hottest known cultivars. *HortScience* **2008**, *43*, 1344–1349.
- (22) Yazawa, S.; Suetome, N.; Okamoto, K.; Namiki, T. Content of capsaicinoids and capsaicinoid-like substances in fruit of pepper (*Capsicum annum* L.) hybrids made with 'CH-19 Sweet' as a parent. *J. Jpn. Soc. Hortic. Sci.* **1989**, *58*, 601–607.
- (23) Yazawa, S.; Ueda, M.; Suetome, N.; Namiki, T. Capsaicinoids content in the fruit of interspecific hybrids in *Capsicum*. *J. Jpn. Soc. Hortic. Sci.* **1989**, *58*, 353–360.
- (24) Kobata, K.; Kawaguchi, M.; Watanabe, T. Enzymatic synthesis of a capsinoid by the acylation of vanillyl alcohol with fatty acid derivatives catalyzed by lipases. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 319–327.
- (25) Kosuge, S.; Murata, M. Studies on the pungent principle of *Capsicum*. Part XIV: chemical constitution of the pungent principle. *Agric. Biol. Chem.* **1970**, *34*, 248–256.
- (26) Zewdie, Y.; Bosland, P. W. Capsaicinoid profiles are not good chemotaxonomic indicators for *Capsicum* species. *Biochem. Syst. Ecol.* **2001**, *29*, 161–169.
- (27) Suzuki, T.; Kawada, T.; Iwai, K. Biosynthesis of acyl moieties of capsaicin and its analogues from valine and leucine in *Capsicum* fruits. *Plant Cell Physiol.* **1981**, *22*, 23–32.
- (28) Stewart, C. Jr.; Kang, B.-C.; Liu, K.; Mazourek, M.; Moore, S. L.; Yoo, E. Y.; Kim, B.-D.; Paran, I.; Jahn, M. M. The *Pun1* gene for pungency in pepper encodes a putative acyltransferase. *Plant J.* **2005**, *42*, 675–688.
- (29) Sutoh, K.; Kobata, K.; Yazawa, S.; Watanabe, T. Capsinoid is biosynthesized from phenylalanine and valine in a non-pungent pepper, *Capsicum annum* L. cv. CH-19 sweet. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 1513–1516.
- (30) Iwai, K.; Suzuki, T.; Fujiwake, H. Formation and accumulation of pungent principles of hot pepper fruits, capsaicin and its analogues in *Capsicum annum* var. *annuum* cv. Karayatsubusa at different growth stages after flowering. *Agric. Biol. Chem.* **1979**, *43*, 2493–2498.
- (31) Contreras-Padilla, M.; Yahia, E. M. Changes in capsaicinoids during development, maturation, and senescence of chile peppers and relation with peroxidase activity. *J. Agric. Food Chem.* **1998**, *46*, 2075–2079.
- (32) Sutoh, K.; Kobata, K.; Watanabe, T. Stability of capsinoid in various solvents. *J. Agric. Food Chem.* **2001**, *49*, 4026–4030.

Received February 24, 2009. Revised manuscript received May 10, 2009.
Accepted May 13, 2009.