Potent production of capsaicinoids and capsinoids by *Capsicum* peppers

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ABSTRACT

The fundamental structure of capsinoids is a fatty acid ester with vanillyl alcohol whereas in capsaicinoids a fatty acid amide is linked to vanillylamine. To clarify the relationship between their biosynthesis in *Capsicum* plants, we carried out an *in vivo* tracer experiment using stable isotopically-labeled putative precursors. LC-MS/MS was used to measure the uptake of isotopes into metabolites after injection of the labeled precursors into intact fruits of a pungent cultivar, Peru, and a nonpungent cultivar, CH-19 Sweet. Labeled vanillylamine was incorporated into capsaicinoids in both cultivars. While labeled vanillyl alcohol was incorporated into capsinoids in both cultivars, the accumulation of intact capsaicinoids in Peru was suppressed by over 60% after administration of vanillyl alcohol. In Peru, labeled vanillin was converted to both vanillylamine and, in 5-fold excess, vanillyl alcohol. Moreover, labeled vanillin was converted exclusively to vanillyl alcohol in CH-19 Sweet. These data are consistent with the incorporation of labeled vanillin into capsaicinoids and capsinoids in both cultivars. We conclude that pungent cultivars are highly potent producers of vanillyl alcohol that is incorporated into capsinoids and that biosynthesis of capsinoids is catalyzed by capsaicin synthase.

KEYWORDS: Capsinoid; capsaicinoid; biosynthesis; vanillin; vanillylamine; vanillyl alcohol; *Capsicum* plants; *in vivo* tracer experiment; stable isotope; LC-MS/MS; putative aminotransferase; capsaicin synthase

INTRODUCTION

The fruits (peppers) of *Capsicum* plants, such as sweet pepper, are used as fresh foodstuffs worldwide, while their processed products are used to make seasonings, including hot peppers, that add color and spice to a variety of cuisines. Moreover, peppers have been traditionally used by certain societies for their stimulatory or analgesic properties. The substances responsible for the stimulatory properties (pungency) of peppers are a group of lipophilic alkaloids, the capsaicinoids. The fundamental structure of capsaicinoids is a branched-chain fatty acid amide of vanillylamine, and the major capsaicinoids in nature are capsaicin (CAP) and dihydrocapsaicin (DC) (**Figure 1**). Capsinoids represent a novel group of nonpungent capsaicinoid-like substances originally found in a nonpungent cultivar, *C. annuum* 'CH-19 Sweet' (*1, 2*), and subsequent studies have shown that many pungent *Capsicum* species contain capsinoids (*3-6*). Capsinoids are defined by a branched-chain fatty acid ester of vanillyl alcohol, and the major naturally occurring capsinoids in nature are capsiate (CST) and dihydrocapsiate (DCT) (**Figure 1**). The chemical structures of these species are similar to those of the major capsaicinoids, with the exception of the center linkage, which is an ester bond in capsinoids and an amide bond in capsaicinoids. Capsinoids have attracted attention for their capsaicinoid-like physiological and biological properties, and their lack of the harmful stimuli of capsaicinoids (*7-9*).

Early classical *in vivo* tracer studies using radiolabeled precursors described the outline of the biosynthetic pathway of capsaicinoids (*10*), in which their aromatic moiety is derived from phenylalanine *via* the phenylpropanoid pathway, and their fatty acid moiety originates from branched amino acids *via* the fatty acid elongation pathway.

Details of the downstream of these pathways however, where vanillin is converted to vanillylamine and the amine is subsequently condensed with a fatty acid to generate a capsaicinoid, remain to be clarified. Recent molecular biological approaches have suggested that the *pAMT* gene of the pungent *Capsicum* fruits encodes a putative aminotransferase that catalyzes the conversion of vanillin to vanillylamine (*11*, *12*). Furthermore, a putative acyltransferase (tentatively referred to as capsaicin synthase, CS) encoded by the *Pun1* gene is regarded as a candidate enzyme catalyzing the condensation of vanillylamine with a fatty acid (*13*-*16*). Given their structural resemblance, the biosynthetic pathways of capsinoids and capsaicinoids are thought to be closely related. A previous *in vivo* tracer study in our group using radiolabeled precursors demonstrated that capsinoids are derived from vanillin *via* vanillyl alcohol in the fruits of CH-19 Sweet (*17*). **Figure 2** shows the proposed pathways for capsaicinoids and capsinoids biosyntheses. Although the conversion of vanillin to vanillyl alcohol and the condensation of the vanillyl alcohol with fatty acid are considered crucial for capsinoids biosynthesis, details regarding these reactions are unclear.

While molecular biological approaches would be complementary in understanding these pathways, defining the flow of metabolites represents a more direct approach to elucidating the biosynthetic pathways of these molecules. To achieve this goal, we describe here *in vivo* tracer experiments using stable isotopically-labeled compounds as putative precursors in the biosynthetic pathways of capsaicinoids and capsinoids. Labeled synthesized precursors were injected into the intact fruits of a capsaicinoid-rich (Peru) and a capsinoid-rich (CH-19 sweet) cultivars, after which the content of labeled metabolites in the fruits were measured using liquid chromatography-tandem mass

spectrometry (LC-MS/MS).

MATERIALS AND METHODS

Materials

Stable isotopically-labeled precursors, [1'-^{13}C][5-^{2}H]-vanillylamine, [1'-^{13}C][5-^{2}H]-vanillyl alcohol, [1'-^{13}C][5-^{2}H]-vanillin, and [1'-^{13}C][5-^{2}H]-ferulic acid, were prepared as described in our previous study (*18*). Other reagents were purchased from Wako Chemicals (Osaka, Japan) and Sigma (St. Louis, MO).

Plants

Capsicum annuum L. cv. Peru (Peru) was used as a capsaicinoid-rich (pungent) cultivar, and *C. annuum* L. cv. CH-19 Sweet (CH-19 Sweet) was used as a capsinoid-rich (nonpungent) cultivar. Plants were cultured in the experiment farm of the University of Shizuoka. Intact green color fruits 16-30 days after anthesis in Peru and 23-30 days in CH-19 Sweet were used for the experiments.

Administration of labeled precursors to intact *Capsicum* fruits

For experiments measuring the conversion of capsaicinoids and capsinoids from labeled precursors, 12.5 μL/day (50 μL in total) of 50 mM labeled precursor in 50 mM potassium phosphate buffer (KPB) solution (pH 6.8) was injected with a micro syringe directly into the loculus of the fruit of an intact plant daily for 4 days. The fruit was harvested a week after the first injection, then frozen in liquid nitrogen and stored at

−20°C until analysis. For time course measurement of metabolites derived from the labeled precursors, 15 µL of 50 mM labeled precursor in KPB was injected into a fruit as described above. The fruit was harvested at 0, 1, 3, and 24 h after the injection, then frozen and stored. The same procedure was followed for control experiments except that KPB only was injected.

Measurements of the metabolites

Stored fruits were freeze-dried and ground individually. To measure the conversion of capsaicinoids and capsinoids from the labeled precursors, the ground fruits were soaked in ethyl acetate. For time course measurement of metabolites, the powdered fruits were soaked in methanol containing 0.1% acetic acid. The supernatant was passed through 0.45 µm pore membrane filter prior to application to an LC-MS/MS system (LC: Nanospace SI-1, Shiseido, Tokyo, Japan; MS/MS: API 2000, Applied Biosystems, Carlsbad, CA).

The LC-MS/MS conditions for measurements of capsaicinoids and capsinoids were as follows: LC column, a reversed-phase silica gel column, Unison UK-C18, 2 mm i.d. x 150 mm (Imtakt Co., Kyoto, Japan); solvent, 50-100% methanol containing 0.1% acetic acid (0-25 min); flow rate, 0.2 mL/min; injection volume, 5 µL; MS/MS, ion source, ESI; polarity, positive; detection mode, multiple reaction monitoring (MRM); detected ions, precursor/product, 306/137 for CAP [M], 308/139 for CAP [M+2], 308/137 for DC [M], 310/139 for DC [M+2], 329/137 for CST [M], 331/139 for CST [M+2], 331/137 for DCT [M], and 333/139 for DCT [M+2]. The ions of CAP, DC, CST, and DCT were observed in the mass chromatogram at 14.5, 16.5, 20.7, and 22.4 min, respectively. The LC-MS/MS conditions for measurements of vanillylamine were

as follows: LC column, a reversed-phase silica gel column, Fluofix 120E, 2 mm i.d. x 150 mm (Wako); solvent, 10-40% methanol containing 0.1% acetic acid (0-20 min); flow rate, 0.2 mL/min; injection volume, 5 µL; MS/MS, ion source, APCI; polarity, positive; detection mode, MRM; detected ions, precursor/product, 137/94 for vanillylamine [M], and 139/96 for vanillylamine [M+2]. The ions of vanillylamine were observed in the mass chromatogram at 5.8 min. The LC-MS/MS conditions for measurements of vanillyl alcohol were as follows: LC column, a reversed-phase silica gel column, Unison UK-C18, 2 mm i.d. x 150 mm; solvent, 10-40% methanol containing 0.1% acetic acid (0-20 min); flow rate, 0.2 mL/min; injection volume, 5 µL; MS/MS, ion source, APCI; polarity, positive; detection mode, MRM; detected ions, precursor/product, 137/94 for vanillyl alcohol [M], 139/96 for vanillyl alcohol [M+2]. The ions of vanillyl alcohol were observed in the mass chromatogram at 9.8 min.

The optimum parameters for the detection of each compound were tuned automatically using authentic samples and Analyst software (Applied Biosystems). The samples were analyzed in duplicate, and each compound was quantified using calibration curves from the authentic samples.

The results quantified by the methods mentioned above were shown in **Tables 1-3** and **Figure 3**. The conversion rates from labeled precursors into capsaicinoids and capsinoids were calculated from the results shown in **Tables 1-3** by an equation below, and were summarized in **Table 4**.

Conversion rate (%) = a molar quantity of peculiarly increased [M+2] products at a week after administration of a precursor / a molar quantity of the administered precursor / 100.

RESULTS

Determination of capsaicinoids, capsinoids, and their precursors

Each administered compound had a vanillyl moiety labeled with deuterium at its aromatic 5-position and ^{13}C at its benzylic position, corresponding to a molecule 2 mass heavier [M+2] than the most abundant natural molecule [M]. Because these labeled positions are present in both capsaicinoids and capsinoids, the labeled compounds can be followed by observing [M+2] molecules. Additionally, both capsaicinoids and capsinoids have typical fragment ions generated by neutral gas collision against their parent ions during mass spectroscopy. The fragment ions originating from the vanillyl moiety of capsaicinoids and capsinoids can be observed in the spectrum at *m/z* 137 for [M] molecules and *m/z* 139 for [M+2] molecules (*18*). In the present study, we measured these metabolites with high selectivity and sensitivity using LC-MS/MS within at least 10 pmol of the quantitation limit, and observed significant alterations of the levels of labeled precursors in intact *Capsicum* fruits. Although the abundance values of [M+2] capsaicinoids and capsinoids (%[M+2]) in control samples measured in this way were lower than the theoretical values, they were stable around 0.7%. Increases in these universal values in intact plants indicate that the labeled precursors have been incorporated into capsaicinoids and capsinoids, and such increases were frequently detected in this study (**Tables 1-3**).

Incorporation of labeled vanillylamine and vanillyl alcohol into capsaicinoids and capsinoids in peppers

Table 1 shows the isotope contents and abundance of capsaicinoids and capsinoids in the fruits of *Capsicum* plants 1 week after administration of [M+2] vanillylamine or [M+2] vanillyl alcohol. High levels of capsaicinoids (CAP and DC) were observed in all samples of the pungent cultivar, Peru. Levels of [M+2] capsaicinoids in Peru plants administered [M+2] vanillylamine were significantly higher than those in control Peru plants. Since the total quantities of the [M] and [M+2] capsaicinoids in both samples were almost equal, the abundance of [M+2] capsaicinoids (%[M+2]) was calculated to be significantly larger in the administered sample than in the control, indicating that [M+2] vanillylamine was incorporated into [M+2] capsaicinoids in Peru. The conversion rate of [M+2] vanillylamine into [M+2] capsaicinoids was estimated at 1% (**Table 4**). The administration of [M+2] vanillyl alcohol in Peru resulted in significant accumulations of [M+2] capsinoids (CST and DCT), with a conversion rate of approximately 2%. Levels of the most abundant form of [M] capsaicinoids in Peru plants administered labeled vannilyl alcohol were significantly suppressed by over 60% compared with those in control and labeled vanillylamine-administered Perus. In the nonpungent CH-19 Sweet cultivar, the administration of [M+2] vanillylamine caused robust accumulation of [M+2] capsaicinoids at an estimated conversion rate of 0.3% (**Table 4**), despite the fact that [M+2] capsaicinoids were undetectable in other CH-19 Sweet samples. Significant increases in the levels of [M+2] capsinoids were observed after administration of [M+2] vanillyl alcohol in CH-19 Sweet, with an estimated conversion rate of 0.5%.

Incorporation of labeled vanillin and ferulic acid into capsaicinoids and capsinoids in peppers

Table 2 shows the effect of administration of [M+2] vanillin in both cultivars. In [M+2] vanillin-administered Peru, levels of [M+2] capsaicinoids and [M+2] capsinoids were higher than those in control Peru plants, with conversion rates of approximately 1.6% and 3.0%, respectively (**Table 4**). On the other hand, the total amounts of capsaicinoids ([M] and [M+2]) in the vanillin-administrated Peru tended to be lower than those in control. Administration of [M+2] vanillin in CH-19 Sweet resulted in significant increases in the levels of [M+2] capsinoids, at an estimated conversion rate of 0.7%. In contrast the conversion rate from vanillin to capsaicinoids was extremely small (0.01%). [M+2] ferulic acid administered in Peru plants was converted to capsaicinoids and capsinoids (**Table 3**) at conversion rates of 0.8% and 0.1%, respectively. In contrast, in CH-19 Sweet, conversion of [M+2] ferulic acid was negligible.

Conversion of labeled vanillin to vanillylamine and vanillyl alcohol in peppers

Figure 3 shows the time course changes of vanillylamine and vanillyl alcohol levels after administration of [M+2] vanillin in Peru and CH-19 Sweet fruits. [M+2] vanillylamine increased immediately after the vanillin administration in Peru, and the maximum level was 36 µg/g dw fruits at 3h post-administration, with a conversion rate estimated at 4%. While vanillyl alcohol was present at only trace levels in control Peru, levels of [M+2] vanillyl alcohol were again significantly increased by 3 h after the vanillin administration to Peru. Maximal levels of [M+2] vanillyl alcohol were 170 µg/g dw, with a conversion rate of 20%, approximately 5 fold higher than in the case of vanillylamine in Peru. The fruits of CH-19 Sweet contained more than 450 µg/g dw of vanillyl alcohol naturally (0 h). Administration of [M+2] vanillin effected an increase of

[M+2] vanillyl alcohol to 80 µg/g dw contents (conversion rate = 9.5%) at 3 h, after which [M+2] vanillyl alcohol was undetectable at 24 h. Negligible [M+2] vanillylamine was observed after administration of [M+2] vanillin into CH-19 Sweet, and native ([M]) vanillylamine was undetectable in samples of CH-19 Sweet.

DISCUSSION

Here we showed that a pungent cultivar of pepper, which predominantly produces capsaicinoids, also produces capsinoids, and that a nonpungent cultivar, which predominantly synthesizes capsinoids, can also produce capsaicinoids. While trace amounts of capsinoids have been previously detected in certain capsaicinoid-producing cultivars (*3-6*), our study represents the first direct observation in peppers of the production of capsinoids from their precursors, with the exception of phenylalanine and valine. In both pungent and nonpungent cultivars, labeled vanillylamine and vanillyl alcohol precursors were incorporated into capsaicinoids and capsinoids, respectively (**Table 1**). Similar results were obtained after administration of vanillin (**Table 2**), which is thought to represent a metabolic junction to vanillylamine or vanillyl alcohol (**Figure 2**). Labeled vanillin was incorporated into both vanillylamine and vanillyl alcohol in the pungent cultivar, Peru (**Figure 3**). The higher conversion of vanillin to vanillyl alcohol than to vanillylamine, which we also observed in a previous radioactive tracer study (*17*), may be due to excess amounts of the external vanillin. The unexpected conversion of vanillin to vanillyl alcohol could conceivably result in the significant production of capsinoids in Peru (**Table 2**). In CH-19 Sweet, incorporation

of labeled vanillylamine into capsaicinoids was less efficient than in Peru, but was nevertheless significant (**Table 1**), while incorporation of labeled vanillin and ferulic acid into capsaicinoids was undetectable (**Tables 2-4)**. Moreover, vanillin was converted to vanillyl alcohol, rather than vanillylamine (**Figure 3**). These results can be explained by the disfunction in CH-19 Sweet of a putative aminotransferase (pAMT) (*19-21*), which catalyzes the conversion of vanillin into vanillylamine. The deficiency of the vanillylamine is likely directly related to the low or non-existent levels of capsaicinoids in CH-19 Sweet.

Another important finding in our study relates to capsaicin synthase (CS), which catalyzes the condensation of vanillylamine with a fatty acid to produce a capsaicinoid (*13-16*). In the present study, accumulation of capsaicinoids in Peru was inhibited by over 60% by the administration of vanillyl alcohol (**Table 1**), prompting speculation that the administered alcohol may compete with native vanillylamine that is normally incorporated into capsaicinoids in a reaction catalyzed by CS. In the case of vanillin administration in Peru, in which both vanillylamine and vanillyl alcohol are produced from the vanillin (**Figure 3**), similar competition was observed (**Table 2**), albeit to a lesser extent. However, we failed to observe inhibition of capsinoid production by the administration of vanillylamine in CH-19 Sweet (**Table 1**). The large vanillyl alcohol pool in intact CH-19 Sweet fruits (**Figure 3)** likely affected our results, in that levels of labeled vanillylamine were insufficient to compete against the endogenous pool of vanillyl alcohol. Moreover, the lower conversion rates of labeled precursors into their end products in CH-19 Sweet compared to Peru (**Table 4)** may be due to the dilution of precursors by this pool. Recently, Han *et al.* reported the potential role of the CS-encoding *Pun1* gene in the biosynthesis of capsinoids in peppers because capsinoids

were present unexceptionally in cultivars of the CS genotype (*6*). CH-19 Sweet is also thought to belong to such a genotype (*20*). Suppression of *pAMT* by gene silencing has been shown to result in significant accumulation of capsinoids in a pungent pepper *(19),* implying that CS catalyzed the production of capsinoids from vanillyl alcohol generated instead of vanillylamine. CS possesses consensus motifs of certain plant acyl-transferases, some of which catalyze the reaction of benzyl alcohol with acyl-CoA to generate the corresponding esters (*15, 22, 23*). In this context, the findings of our metabolic flow study provide additional evidence supporting the contribution of CS to capsinoid biosynthesis.

The rate of conversion of labeled vanillyl alcohol to capsinoids was approximately double that of the conversion of labeled vanillylamine to capsaicinoids in both cultivars (**Table 4**). A similar tendency was observed in the case of vanillin administration in Peru. Given that labeled vanillin was predominantly converted to vanillyl alcohol in Peru (**Figure 3**), the potential of CS for production of capsinoids may be similar to that for production of capsaicinoids. We speculate therefore that the relative level of capsaicinoids and capsinoids is a function of the levels of their direct precursors, vanillylamine and vanillyl alcohol, respectively. The factors determining the conversion of vanillin to vanillyl alcohol in peppers is not known at present.

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Figure Captions

Figure 1. Chemical structures of capsaicinoids (capsaicin and dihydrocapsaicin) and capsinoids (capsiate and dihydrocapsiate).

Figure 2. Proposed biosynthetic pathways of capsaicinoids and capsinoids. pAMT, putative aminotransferase; CS, capsaicin synthase.

Figure 3. The time course change of contents of vanillylamine and vanillyl alcohol in the fruits of peppers after administration of [1'-^{13}C][5-^{2}H]-vanillin. Data are shown as means ± S.E.M.

Table 1

Isotope ([M], [M+2]) contents (μg/g dw fruits) and abundance (%[M+2]) of capsaicinoids and capsinoids in the fruits of peppers cultivated with [1'-^{13}C][5-^{2}H]-vanillylamine (+ VNH_2), with [1'-^{13}C][5-^{2}H]-vanillyl alcohol (+ VOH), or without any precursor (Cont.) for a week.

		Peru			CH-19 Sweet		
		Cont. (n=3)	+ VNH_2 (n=5)	+ VOH (n=6)	Cont. (n=6)	+ VNH_2 (n=5)	+ VOH (n=6)
CAP	[M]	2213.6±238.8^{a}	1904.3±208.4^{a}	796.6±94.2^{b}	5.2±0.5^{a}	7.8±0.3^{b}	6.1±0.9^{ab}
	[M+2]	15.5±1.6^{a}	38.3±6.2^{b}	8.6±1.1^{a}	nd	3.0±1.4	nd
	(%[M+2])	(0.70±0.01)^{a}	(2.18±0.52)^{b}	(1.12±0.11)^{ab}	(nc)	(37.53±16.91)	(nc)
DC	[M]	1250.2±154.2^{a}	1179.8±131.2^{a}	444.3±48.8^{b}	5.7±0.6^{a}	7.7±0.4^{b}	6.2±0.5^{ab}
	[M+2]	9.7±1.0^{a}	45.2±10.5^{b}	8.7±1.1^{a}	nd	6.9±2.6	nd
	(%[M+2])	(0.78±0.03)^{a}	(4.13±1.16)^{b}	(2.04±0.26)^{ab}	(nc)	(98.63±42.09)	(nc)
CST	[M]	304.2±49.3^{a}	387.6±13.7^{a}	129.1±26.9^{b}	1080.9±73.9^{a}	1291.8±55.2^{a}	1100.8±110.7^{a}
	[M+2]	nd	nd	86.4±16.5	7.4±0.7^{a}	8.8±0.6^{a}	14.6±2.3^{b}
	(%[M+2])	(nc)	(nc)	(72.87±17.55)	(0.68±0.02)^{a}	(0.68±0.03)^{a}	(1.30±0.11)^{b}
DCT	[M]	38.1±15.1^{a}	112.6±14.8^{b}	35.2±8.4^{a}	318.8±34.4^{a}	336.9±65.5^{a}	400.4±56.8^{a}
	[M+2]	nd	nd	31.3±6.5	nd	nd	7.6±1.2
	(%[M+2])	(nc)	(nc)	(89.07±24.43)	(nc)	(nc)	(1.93±0.23)

CAP: capsaicin, DC: dihydrocapsaicin, CST: capsiate, DCT: dihydrocapsiate

nd: not detected, nc: not calculated

Data are shown as means ± S.E.M.

Different letters indicate significant differences (Tukey's multiple-comparison test, $P<0.05$).

Table 2

Isotope ([M], [M+2]) contents (µg/g dw fruits) and abundance (%[M+2] of capsaicinoids and capsinoids in the fruits of peppers cultured with [1'-^{13}C][5-^{2}H]-vanillin (+ V) or without any precursor (Cont.) for a week.

		Peru		CH-19 Sweet	
		Cont. (n=3)	+ V (n=5)	Cont. (n=6)	+ V (n=5)
CAP	[M]	2294.2±266.8	1769.5±243.2	5.4±0.4	7.1±1.9
	[M+2]	16.2±2.0	34.7±2.8*	nd	0.2±0.1
	(%[M+2])	(0.70±0.01)	(2.28±0.62)	(nc)	(2.69±1.23)
DC	[M]	1613.1±150.2	1111.2±144.6	4.5±0.4	6.8±1.6
	[M+2]	11.7±1.0	36.7±3.7*	nd	0.4±0.1
	(%[M+2])	(0.73±0.02)	(3.70±0.87*)	(nc)	(6.29±0.89)
CST	[M]	247.7±46.1	192.1±33.3	1230.1±87.1	1593.8±129.3*
	[M+2]	nd	76.7±15.3	8.7±0.9	30.9±4.6*
	(%[M+2])	(nc)	(52.26±19.70)	(0.71±0.03)	(1.92±0.20*)
DCT	[M]	23.1±8.9	24.0±9.5	360.2±45.2	720.9±17.9*
	[M+2]	nd	31.0±18.1	nd	16.9±2.9
	(%[M+2])	(nc)	(104.04±21.43)	(nc)	(2.35±0.43)

CAP: capsaicin, DC: dihydrocapsaicin, CST: capsiate, DCT: dihydrocapsiate

nd: not detected, nc: not calculated

Data are shown as means ± S.E.M.

Significant differences (Student's t-test, * $P<0.05$) against control (Cont.)

Table 3

Isotope ([M], [M+2]) contents (μg/g dw) and abundance (%[M+2]) of capsaicinoids and capsinoids in the fruits of peppers cultured with [1'-^{13}C][5-^{2}H]-ferulic acid (+ FA) or without any precursor (Cont.) for a week.

		Peru		CH-19 Sweet	
		Cont. (n=6)	+ FA (n=7)	Cont. (n=6)	+ FA (n=6)
CAP	[M]	2004.6±72.0	1710.5±108.9	5.0±0.6	6.7±0.4*
	[M+2]	8.9±0.7	15.7±1.9	nd	nd
	(%[M+2])	(0.64±0.01)	(0.91±0.09*)	(nc)	(nc)
DC	[M]	1186.4±89.4	1085.6±64.5	5.0±0.5	9.2±0.2*
	[M+2]	9.8±0.6	23.9±4.3*	nd	nd
	(%[M+2])	(0.75±0.01)	(2.18±0.38*)	(nc)	(nc)
CST	[M]	240.0±15.7	261.5±22.3	1100.0±93.8	1174.9±90.5
	[M+2]	nd	4.8±1.3	6.8±0.8	8.2±1.7
	(%[M+2])	(nc)	(1.95±0.56)	(0.62±0.03)	(0.68±0.09)
DCT	[M]	26.5±1.6	26.3±2.0	408.5±56.1	698.8±73.6*
	[M+2]	nd	nd	nd	nd
	(%[M+2])	(nc)	(nc)	(nc)	(nc)

CAP: capsaicin, DC: dihydrocapsaicin, CST: capsiate, DCT: dihydrocapsiate

nd: not detected, nc: not calculated

Data are shown as means ± S.E.M.

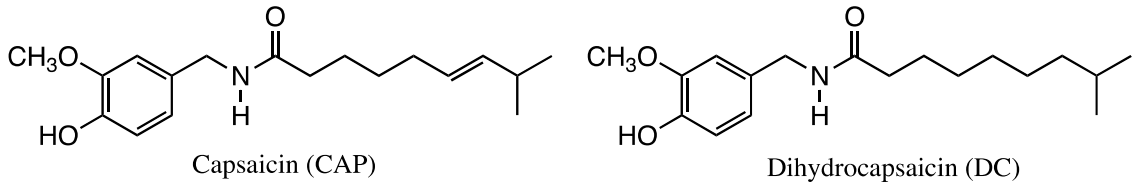
Significant differences (Student's t-test, * $P<0.05$) against control (Cont.)

Table 4
Conversion rate (%) from administered precursors to capsaicinoids (CAPs) and capsinoids (CSTs) for a week.

	Peru (n=5-7)	CH-19 Sweet (n=5-6)
VNH_2 → CAPs	0.97±0.25	0.29±0.09
VOH → CSTs	1.97±0.47	0.54±0.09
V → CAPs	1.56±0.18	0.01±0.00
V → CSTs	3.04±0.82	0.69±0.15
FA → CAPs	0.84±0.21	0.00±0.00
FA → CSTs	0.12±0.05	0.03±0.05

CAPs: capsaicin + dihydrocapsaicin, CSTs: capsiate + dihydrocapsiate, VNH_2: vanillylamine, VOH: vanillyl alcohol, V: vanillin, FA: ferulic acid

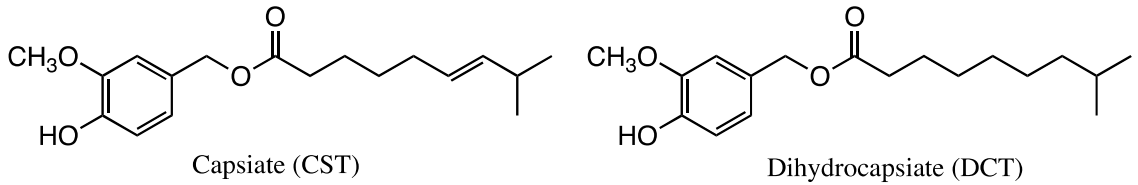
Figure 1



Capsaicin (CAP)

Dihydrocapsaicin (DC)

Capsaicinoids



Capsiate (CST)

Dihydrocapsiate (DCT)

Capsinoids

Figure 2

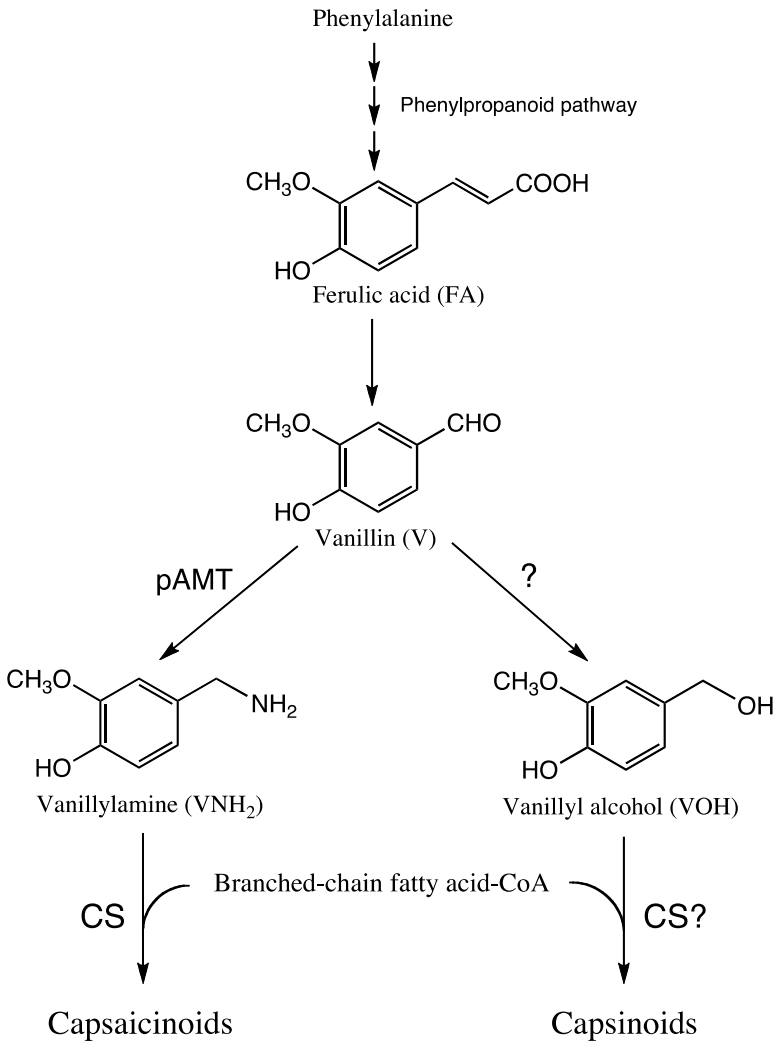
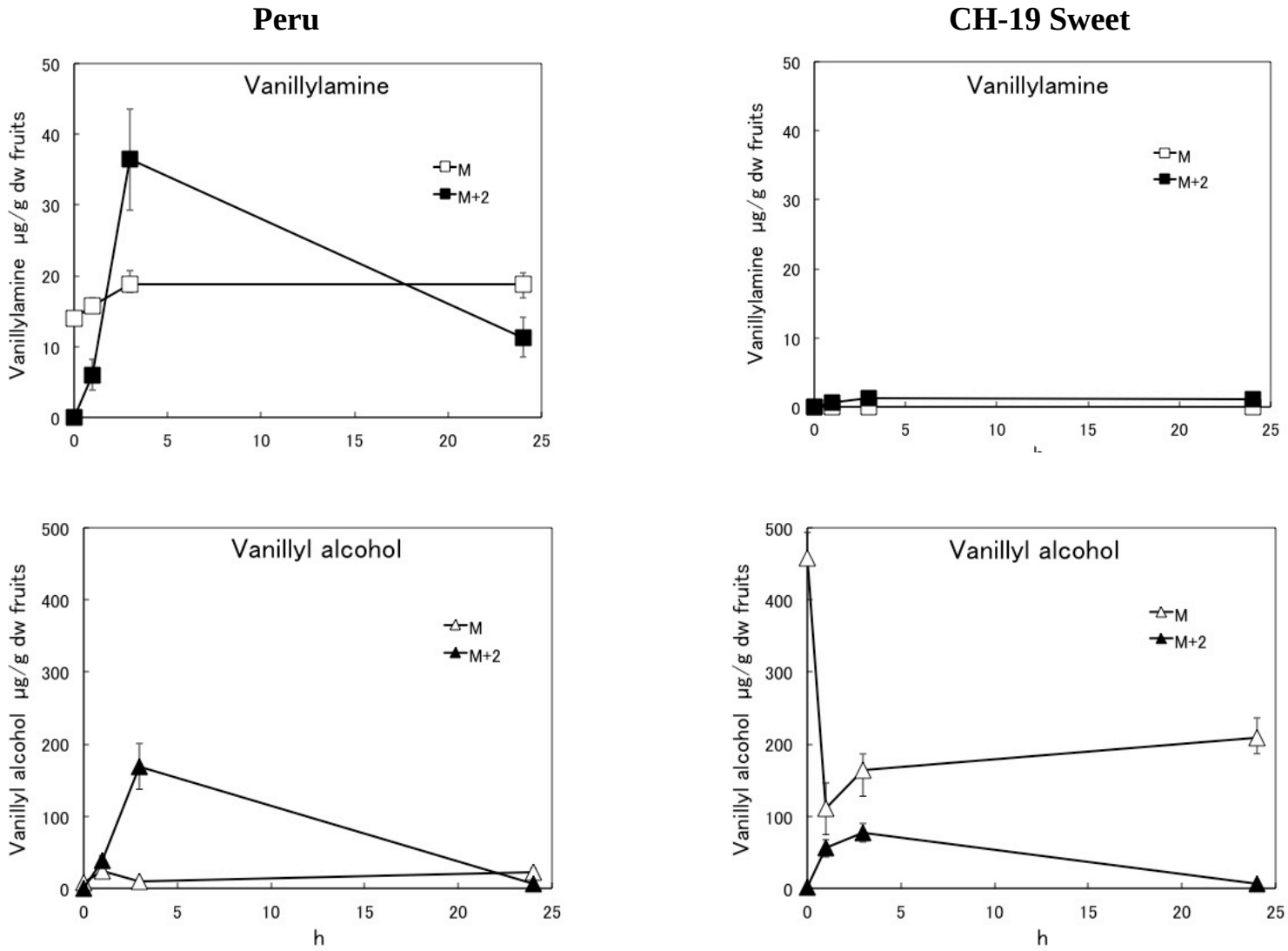
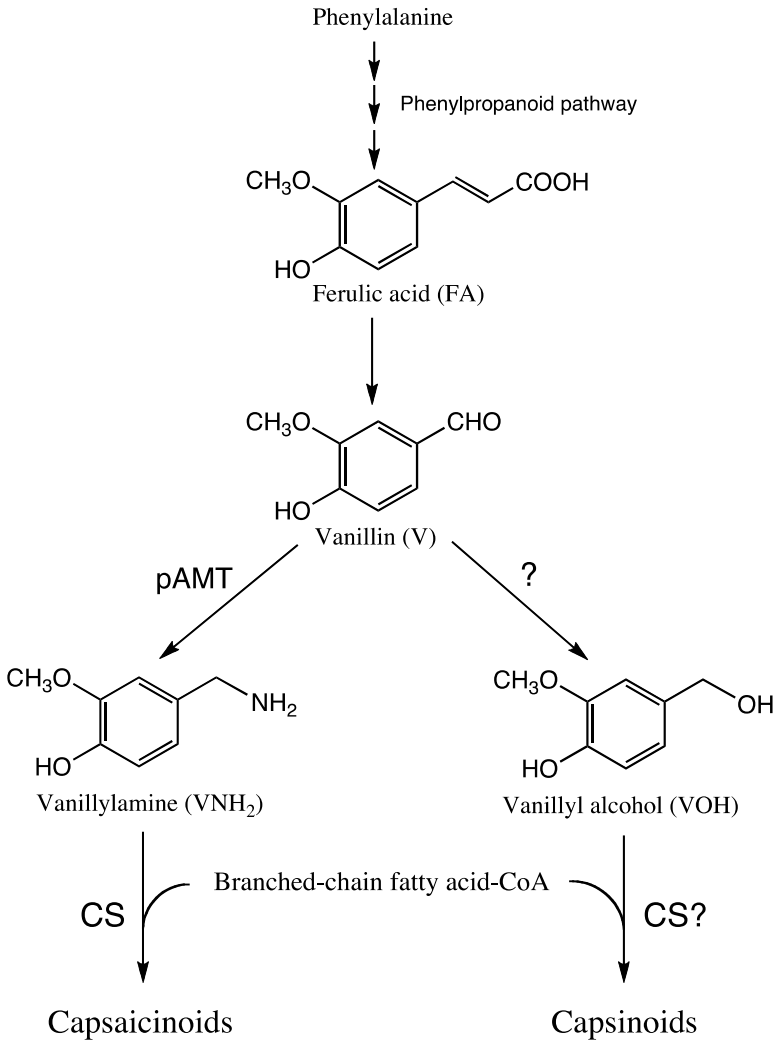

Phenylalanine
Phenylpropanoid pathway
CH3O
COOH
HO
Ferulic acid (FA)
CH3O
CHO
HO
Vanillin (V)
pAMT
?
CH3O
NH2
HO
Vanillylamine (VNH2)
CH3O
OH
HO
Vanillyl alcohol (VOH)
Branched-chain fatty acid-CoA
CS
CS?
Capsaicinoids
Capsinoids

Figure 3


Peru
CH-19 Sweet
Vanillylamine
Vanillylamine µg/g dw fruits
M
M+2
Vanillyl alcohol
Vanillyl alcohol µg/g dw fruits
h

TOC Graphic


Phenylalanine
Phenylpropanoid pathway
CH3O
COOH
HO
Ferulic acid (FA)
CH3O
CHO
HO
Vanillin (V)
pAMT
?
CH3O
NH2
HO
Vanillylamine (VNH2)
CH3O
OH
HO
Vanillyl alcohol (VOH)
Branched-chain fatty acid-CoA
CS
CS?
Capsaicinoids
Capsinoids