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Capsaicin and Dihydrocapsaicin Contents of Thai Chili Cultivars

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ABSTRACT

Ten cultivars of Thai chilies, including six *Capsicum annuum* L. and four *C. frutescens* L. were grown under field conditions to determine their capsaicin and dihydrocapsaicin contents using high-performance liquid chromatography. The capsaicin content of the chili cultivars ranged from 0.76-3.76 mg/g and the dihydrocapsaicin content ranged from 0.59-2.39 mg/g. One *C. frutescens* cultivar (K07) and two *C. annuum* cultivars (Huayseeton SK1 and Huarua) had high capsaicin contents, while two *C. annuum* cultivars (Huarua and Pijit007) had high dihydrocapsaicin contents. The Huarua, Huayseeton SK1 and K07 cultivars had the highest capsaicinoid contents which were greater than 5.2 mg/g.

The Thai chili cultivars in this study, with the exception of K05, were classified as 'highly pungent' with a pungency range from 45,000-80,000 Scoville Heat Units (SHU). The Huarua and K07 cultivars were classified as 'very highly pungent' chilies with SHU values greater than 80,000. All chili cultivars however, had a higher content of capsaicin than of dihydrocapsaicin. High capsaicin to dihydrocapsaicin ratios were found in the K07 and Huayseeton SK1 cultivars. Moreover, capsaicin and dihydrocapsaicin contents were highest in the first harvest in all cultivars and decreased in subsequent harvests.

Key words: *Capsicum annuum* L., *Capsicum frutescens* L., capsaicinoids, pungency

INTRODUCTION

Chili is grown worldwide as a vegetable and a spice and is generally recognized as being either pungent or non-pungent. In Thailand pungent chili is an economically important crop grown for local consumption and for domestic and international food industry markets.

Pungency, a commercially important attribute of chili, is due to the presence of alkaloid compounds in the fruit known as capsaicinoids (Hoffman *et al.*, 1983). The two most abundant capsaicinoids in chilies are capsaicin and dihydrocapsaicin, accounting for 69 and 22% respectively of the total capsaicinoids in most of the pungent varieties (Kosuge and Furuta, 1970). The degree of pungency varies among species and varieties (Singh *et al.*, 2003). The capsaicin and dihydrocapsaicin contents can be affected by different factors such as the developmental stage

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of the fruit (Sukrasno and Yeoman, 1993) and the environmental conditions (Zewdie and Bosland, 2000a). Capsaicinoid compounds have been widely studied and are currently used in the food industry, for medical purposes and in defensive sprays.

Chilies in Thailand are of the cayenne fruit type, typically with two fruit sizes, long and short. The length of long chilies ranges from 9-15 cm and the pungency ranges from very low to medium-hot, while the length of short chilies ranges from 2-7 cm and the pungency ranges from medium to very hot. The species of short chilies may be either *Capsicum annuum* L. or *Capsicum frutescens* L. (Berke, 2002). In Thailand, short chilies are named ' Keenoo' and have the biggest production area (Lerdrat, 2007). Differences in plant habit, corolla color and number of flowers per node allow easy discrimination of the two species. *C. annuum* is an annual plant with white flowers and one flower per node, while *C. frutescens* is a perennial with greenish flowers and 1-2 flowers per node (Greenleaf, 1986).

The climate in Thailand is ideal for chili production and over production frequently leads to low market prices for fresh chilies. Selectively growing chili varieties that differ in their pungency (capsaicinoid content) would add value to the crop and would allow them to be marketed on the basis of their pungency. The objective of this study was to determine the capsaicin and dihydrocapsaicin contents from early to late harvest in high-yield Kenoo chili cultivars commonly grown in Thailand.

MATERIALS AND METHODS

Plant materials

Ten cultivars of Thai chilies, including six *C. annuum* and four *C. frutescens* cultivars were grown at the Kanchanaburi Horticultural Research Center from January to September during 2004. *C. annuum* was planted 50 cm apart in rows spaced 100 cm apart (4 rows; 28 plants/cultivar), while *C. frutescens* was planted in a grid with plants spaced 100 cm (4 rows; 16 plants/cultivar). The experiment was conducted in a randomized complete block design with three replicates. The *C. annuum* cultivars included Pijit 007, Pijit 0014, Pijit 0054, Huayseeton SK1 (Huayseeton Srisaket 1), Huarua and Jinda. The *C. frutescens* cultivars included K04 (Kanchanaburi 04), K05 (Kanchanaburi 05), K07 (Kanchanaburi 07) and K09 (Kanchanaburi 09) (Figure 1).

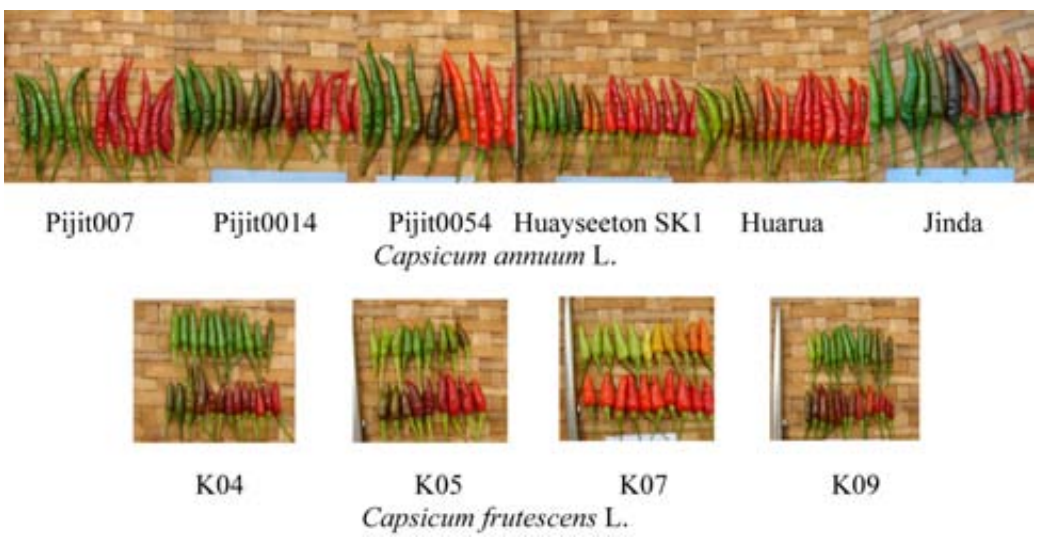

Figure 1 Six cultivars of *Capsicum annuum* L. and four cultivars of *Capsicum frutescens* L.

Determination of capsaicin and dihydrocapsaicin contents

The capsaicin and dihydrocapsaicin content of ten chili cultivars was determined once a month over a three month period. Fruits of *C. frutescens* were harvested two months later than the *C. annuum* fruits and the first harvest of the latter species was available in April. Fully ripening fruits of each cultivar were harvested three times at 30-day intervals, with 40 fruits of *C. annuum* and 80 fruits of *C. frutescens* being picked.

Chili preparation and capsaicin and dihydrocapsaicin extraction

The fruit pedicels and seeds were removed and separated from the flesh (ovary wall and placenta). The flesh was then dried at 50°C for 2-4 days and ground to powder. Five grams of the chili powder was then soaked in 50 ml acetone and shaken at 150 rpm for six hours at room temperature (25°C). The chili-acetone mixture was vacuum-filtrated and only the clear supernatant was analyzed. The acetone was evaporated to obtain crude extract which was then diluted in 50 ml methanol and filtrated using a 0.45 μm syringe filter (Prapanoppasin, 2001). Two ml of the supernatant was transferred to a vial test tube for analysis.

Capsaicin and dihydrocapsaicin content analysis

The capsaicin and dihydrocapsaicin contents were analyzed using high performance liquid chromatography (HPLC; series HP1100 Agilent) equipped with a Luna C_{18} column (5 μ, 50 × 4.6 mm) and a UV detector at 284 nm. The HPLC analysis was carried out at 25°C. The mobile phase was a mixture of methanol and water (65:35 v/v) and the flow rate was 1ml/min (Collins *et al*., 1995). The retention times were 7.5 min for capsaicin and 11.0 min for dihydrocapsaicin.

The capsaicin and dihydrocapsaicin in each sample were identified and quantified by comparison with 98% pure capsaicin and 90% pure dihydrocapsaicin standard compounds (Sigma). Standard curves were prepared using serial dilutions of 25, 50, 100, 200, 400 and 800 mg/l for capsaicin and 12.5, 25, 50, 100, 200 and 400 mg/l for dihydrocapsaicin. The pungency level in Scoville Heat Units (SHU) was calculated by using the capsaicinoids content in ppm × 15 (Collins *et al*., 1995).

RESULTS AND DISCUSSION

Capsaicin and dihydrocapsaicin contents

The capsaicin contents ranged between 0.76 - 3.76 mg/g and the dihydrocapsaicin contents ranged from 0.59 - 2.39 mg/g. The K07 cultivar had the highest capsaicin content (3.76 mg/g) followed by Huayseeton and Huarua (3.60 and 3.42 mg/g, respectively) while Huarua had the highest dihydrocapsaicin content followed by Pijit007 (2.39 and 2.06 mg/g, respectively). The lowest capsaicin and dihydrocapsaicin contents however were found in K05 (Table 1).

Capsaicinoid content

The capsaicinoid content was calculated from the sum of the capsaicin and dihydrocapsaicin contents in each cultivar (Table1). Nine of the assayed chili cultivars had total capsaicinoid contents ranging from 3.42 to 5.81 mg/g. The exception to this pattern was K05 (*C. frutescens*), which had a very low capsaicinoid content of 1.35 mg/g**.** Three of *C. annuum* cultivars (Huarua, Huayseeton and SK1) and one of the *C. frutescens* cultivars (K07), had particularly high total capsaicinoid contents of greater than 5.2 mg/g. Generally, *C. frutescens* has been reported as a highly pungent chili (Greenleaf, 1986; Bosland and Votava, 1999). However, K05, a *C. frutescens* cultivar, had the lowest capsaicin content of all cultivars examined. This could have been due to the K05 cultivar having a thick pericarp and a small placenta compared to the other *C. frutescens* cultivars, which had a thin pericarp with a large portion of placenta which would be consistent with the observation of Balbaa *et al*. (1968) that the fruits with thinner pericarps are generally more pungent than fruits with thicker pericarps.

All chili cultivars except Huayseeton SK1 and K07 contained 1.18-1.89 times more capsaicin than dihydrocapsaicin the two cultivars with high capsaicin contents. Huayseeton SK1 and K07 had twice as much capsaicin as dihydrocapsaicin, while K05, the least pungent chili cultivar, had equal contents of capsaicin and dihydrocapsaicin (Table1).

Kosuge *et al*., (1961) reported the ratio of capsaicin to dihydrocapsaicin from *C annuum* extracts was 7:3 while a ratio of about 2:1 was reported in *C. frutescens* (Zewdie and Bosland, 2001). This difference may have been due to the cultivar, environmental conditions, fruit maturation or post-harvest factors (Zewdie and Bosland, 2000a). These result confirmed that the pungency level depended on the cultivar and fruit morphology and not on the species of chili.

There are five pungency levels classified using Scoville Heat Units (SHU): non-pungent (0-700 SHU), mildly pungent (700-3,000 SHU), moderately pungent (3000-25,000 SHU), highly pungent (25,000-70,000 SHU) and very highly pungent (> 80,000 SHU) (Weiss, 2002). The use of SHU is the traditional method to evaluate chili pungency, as it provides a better indicator of the pungency level than the HPLC method but is less precise (Collins *et al*., 1995).

All Thai chili varieties in this study except for K05 were classified as highly pungent, with their pungency ranging from 50,000- 80,000 SHU (Table 1). Huarua and K07 cultivars were classified as having the highest pungency, with more than 80,000 SHU. But based only on the capsaicin content, which is the criterion used by the pharmaceutical industry, K07 (3.76 mg/g) and Huayseeton SK1 (3.60 mg/g) would be preferred by this industry. However, Huayseeton SK1 has more potential for this purpose, because it has been identified as a higher-yield cultivar (about 2,500 kg/rai) (DOA, 1998) than K07 (about 960 kg/rai) (Kraikruan *et al*., 2000). This cultivar was also able to be harvested earlier than K07, starting at three months after transplanting. Huayseeton SK1 should thus be considered as a capsaicin cultivar for commercial production.

Table 1 Average capsaicin (CAP), dihydrocapsaicin (DHCAP), capsaicinoid (CAPN) contents, CAP: DHCAP ratio and pungency level of ten Thai chili cultivars harvested once a month over a three month period.

Species/Cultivar	CAP (mg/g DW)	DHCAP (mg/g DW)	CAP:DHCAP	CAPN (mg/g DW)	Pungency level[1/] (SHU)
Capsicum annuum					
Pijit 007	2.44 ± 0.035e[2/]	2.06 ± 0.071b	1.18	4.50 ± 0.038d	67,425
Pijit 0014	2.82 ± 0.062d	1.65 ± 0.069c	1.72	4.47 ± 0.046d	67,035
Pijit 0054	2.12 ± 0.039g	1.48 ± 0.094e	1.43	3.59 ± 0.110g	53,910
Huarua	3.42 ± 0.046c	2.39 ± 0.036a	1.43	5.81 ± 0.078a	87,135
Huayseeton SK1	3.60 ± 0.011b	1.60 ± 0.045cde	2.25	5.21 ± 0.056c	78,075
Jinda	2.09 ± 0.077g	1.33 ± 0.134f	1.57	3.42 ± 0.059h	51,255
C. frutescens					
K04	2.84 ± 0.005d	1.50 ± 0.010de	1.89	4.34 ± 0.008e	65,100
K05	0.76 ± 0.013h	0.59 ± 0.044g	1.29	1.35 ± 0.034i	20,250
K07	3.76 ± 0.027a	1.63 ± 0.057cd	2.31	5.39 ± 0.060b	80,805
K09	2.28 ± 0.070f	1.60 ± 0.065cde	1.43	3.88 ± 0.061f	58,260
CV(%)	1.4	4.7		1.4	

[1/] SHU = ppm of capsaicinoids x15

[2/] Means in the same columns followed by the same letter are not significantly different at P<0.05 level by Duncan's Multiple Range Test (DMRT)

Changes of capsaicin and dihydrocapsaicin content during the harvesting period

The capsaicin and dihydrocapsaicin contents of all the chili varieties decreased significantly from early to late harvest (Table 2). The highest capsaicin and dihydrocapsaicin contents were obtained during the first harvest (April for *C. annuum* and June for *C. frutescens*) and subsequently decreased in later harvests.

Capsaicinoid content in chili is genetically controlled, but its variation is significantly affected by growing conditions (Harvell and Bosland, 1997). High temperature has been reported to increase the capsaicinoid content (Sung *et al*., 2005). In this study, the decline in capsaicinoid content throughout the harvesting period seemed to be related to plant age. The high pungency in young plants may have been related to the number of fruits. In the early developmental stage, there were fewer fruits per plant and so competition between fruits for nutrients to produce capsaicinoids may have been minimal (Sukrasno and Yeoman, 1993). However, at later growth stages, there were more fruits per plant and competition may have been higher, resulting in a low amount of capsaicinoid production. A similar pattern was reported in *C. annuum* , with a decrease in pungency on fruit at the higher nodes (Zewdie and Bosland, 2000b). Therefore, harvesting for high capsaicinoid content should occur early in the picking season during the first two month period, unless the application of growth-promoting factors (water, fertilizer, pruning *etc*.) is adequate for the whole growing season.

CONCLUSIONS

The average capsaicin contents of ten chili cultivars ranged from 0.76-3.76 mg/g and the dihydrocapsaicin contents ranged from 0.59 - 2.39 mg/g. The cultivars K07, Huayseeton SK1 and Huarua had the highest capsaicin contents, while Huarua and Pijit 007 had the highest dihydrocapsaicin contents. Three cultivars, Huarua, Huayseeton SK1 and K07 had the highest capsaicinoid content, which was greater than 5.2 mg/g. Most chili cultivars contained around 1.18-1.89 times more capsaicin than dihydrocapsaicin, while Huayseeton SK1 and K07 had twice as much capsaicin as dihydrocapsaicin. The highest capsaicin and dihydrocapsaicin contents of each cultivar were found in the first harvest and decreased in subsequent harvests.

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Table 2 Capsaicin and dihydrocapsaicin contents of Thai chili cultivars harvested during the first, second and third month.

Harvesting time	Pijit 007	Pijit 0014	Pijit 0054	□ Huarua	Huayseeton SKI	Jinda	K04	K05	K07	K09	Mean
					Capsaicin (mg/g)						
1st	2.73	3.66	3.02	4.18	4.33	2.45	3.95	1.22	4.85	2.99	3.34 a[1/]
2nd	2.45	2.23	1.90	3.56	4.30	1.99	2.85	0.59	3.71	2.61	2.62 b
3rd	2.12	2.58	1.43	2.51	2.18	1.82	1.71	0.47	2.72	1.24	1.24 c
CV(%)	-	-	-	-	-	-	-	-	-	-	16.8
					Dihydrocapsaicin (mg/g)						
1st	2.20	1.73	2.03	2.46	2.20	1.58	1.96	0.87	1.90	1.20	1.89 a
2nd	2.19	2.11	1.26	2.93	1.78	1.34	1.64	0.48	1.72	1.71	1.72 a
3rd	1.80	1.10	1.13	1.79	0.84	1.08	0.91	0.42	1.26	1.10	1.42 b
CV(%)	-	-	-	-	-	-	-	-	-	-	15.1

[1/] Means in the column followed by the same letter are not significantly different at P<0.05 level by DMRT

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