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Research Article

Diversity in Capsaicin and Dihydrocapsaicin Content in Hot Pepper Genotypes

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Abstract

Twenty-six *Capsicum* genotypes that represent four hot pepper species, *Capsicum annuum* L., *C. baccatum* L., *C. chinense* Jacq., and *C. frutescens*, were screened for their capsaicin and dihydrocapsaicin content. The main objective of this investigation was to identify pepper genotypes that have high concentrations of capsaicin and dihydrocapsaicin in fresh mature fruits for Kentucky growers who are seeking specialty cash crop comprising health promoting properties. Capsaicin and dihydrocapsaicin were extracted using methanol, purified using microfiber disks, and quantified using a gas chromatograph equipped with a nitrogen phosphorus detector (GC/NPD). Analysis of fruit extracts using mass spectrometry indicated the incidence of two molecular fragments at m/z 305 and 307 that match capsaicin, and dihydrocapsaicin, respectively. The results revealed that plant identification (PI) 631144 from *C. frutescens* contained the greatest concentration of capsaicin (323 μg g^{-1} fresh fruit), whereas PI 123474 from *C. annuum* contained the greatest concentrations of dihydrocapsaicin (205 μg g^{-1} fresh fruit).

INTRODUCTION

Hot pepper fruits (*Capsicum* spp.) have been used worldwide to enhance the food flavor due to their sensory and health benefit properties. The USDA *Capsicum* collection contains thousands of accessions of *Capsicum* spp., while only limited information is currently available on their internal composition. The genus *Capsicum* (Family: Solanaceae) contains five commonly cultivated species (*C. annuum* L., *C. frutescens* L., *C. chinense* Jacq., *C. baccatum* L. and *C. pubescens* Ruiz & Pav.). They show varying degrees of pungency that reflect the levels of capsaicin [N-vanillyl-8-methyl-6-nonenamide], dihydrocapsaicin, and other analogs [1,2] that are known collectively as capsaicinoids [3]. Capsaicin in this group exhibits antioxidant activity, antimutagenic and anticarcinogenic properties [4]. Concentrations of total capsaicinoids are about 80-95% in peppers [5,6]. Nordihyrocapsaicin, homocapsaicin, and homodihydrocapsaicin are other capsaicin analogs generally present in trace amounts.

Pungency in hot pepper is considered an important fruit quality attribute due to fruit health-promoting properties [7] and a variety of medicinal uses [8]. There is a growing interest in the development of compounds in foods having health-endorsing features [9, 10]. Capsaicin in hot pepper creates a desirable spice and a valued international commodity [11]. The U.S. chili pepper production occurs mainly in New Mexico, eastern Arizona, and western Texas [12] with *C. chinense* as the most cultivated pepper in South America [11]. Fruits of this species are generally quite pungent. Scotch Bonnet and Habanero-type peppers are regarded as examples of the extremes in pungent pepper present in cultivated forms of *C. chinense* [13] and other species. The concentration of capsaicinoids and the proportion of capsaicin/dihydrocapsaicin varies within and among species [14]. Capsaicinoids concentration varies due to a variety of environmental, cultural, and other factors [15].

Huntrods [16] reported that the consumption of peppers has increased during the last decade. In the U.S. alone, pepper consumption has increased about 8% in the 2000-2007 period and according to the USDA Economic Research Service, [17] most of the increase in consumption was related to consumption of specialty peppers. Consumption of Chile peppers in the U.S. is now more than 6 lbs. per person. Imports are increasing disseminated in the Chile market, accounting for 72% of domestic supply in 2003-2005. While these data include both fresh and processed Chile peppers, between 2002 and 2005 there was a 41% increase in the value of fresh Chile peppers imported into the U.S. In the U.S., consumption of Chile peppers is increasing as fresh product. There are probably multiple reasons for increased consumption of specialty peppers. Consumption of ethnic foods continues to increase, related to increases in ethnic populations in the U.S. and a greater interest in ethnic foods in the general U.S. population. Chile peppers may hold a special interest in a subset of the

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U.S. population. A Google search for Chile pepper production, showed more than 1,300,000 hits. Clearly, the trend is positive for consumption of specialty peppers. All these popularity have helped to drive spicy pepper consumption and prices of local produce [18].

There is a direct correlation between total capsaicinoids level and pungency as measured in Scoville heat units (SHU). SHU scale is a measurement of the pungency (spicy heat) of chili peppers—such as the jalapeño, the hut jolokia, and the world's current hottest pepper.

There are five levels of pungency classified using: non-pungent (0–700 SHU), mildly pungent (700–3,000 SHU), moderately pungent (3,000–25,000 SHU), highly pungent (25,000–70,000 SHU) and very highly pungent (>80,000 SHU) [19]. Today, the SHU organoleptic test has been replaced by chromatographic methods which are found to be more consistent and accurate compared to the SHU scale. Capsaicinoids content is a major quality factor in spice (Chile and paprika) peppers. Accordingly, variability in the content of capsaicinoids greatly impacts pepper pungency. Hot pepper producers look for varieties that yield high quality peppers. Characteristics of interest included yield, fruit size and shape, wall thickness, and plant size [20].

According to Bosland and Votava [21], the consumption and public interest for pepper is increasing. Food producers have become more interested in growing new crops to meet the growing demands of consumers who are looking for food with health promoting properties [9]. There are thousands of different pepper varieties around the world, making their identification, plant and fruit variations challenging.

The present investigation is a continuation of the author's work on hot pepper production and was conducted to: 1) examine twenty-six previously uncharacterized genotypes of *Capsicum* species selected from the USDA *Capsicum* collection for variability in fruit concentrations of capsaicinoids (capsaicin and dihydrocapsaicin) that might be utilized through genetic selection and manipulations to enhance pepper fruit content of capsaicinoids for use as a cash crop and medicinal agent.

MATERIALS AND METHODS

Field description

Seeds of four hot pepper species, identified in the genebank inventory as Plant Identification (PI) of *Capsicum spp.* were obtained from the USDA and sown in the greenhouse at the University of Kentucky South Research Farm. Seedlings of 120 day-old of these genotypes were transplanted in the field (Figure 1) on May 20, 2015 in a randomized complete block design (RCBD) into rows about 1 m apart and 0.25 m between plants within rows. Thirty-cm of extra space was allowed between soil treatments and the plants were fertilized with Peters (200 ppm) of the elements NPK (20:20:20) and weeded as needed. Plants were grown in raised beds with black plastic mulch and drip irrigation system. The selected genotypes were: Twelve *Capsicum annuum* (PI 123474, PI 127442, PI 138565, PI 159256, PI 164271; PI 169129, PI 200725, PI 210980, PI 215743, PI 241670, PI 246331, PI 257048); three *Capsicum baccatum* (PI 260539, PI 260571, PI 439409); ten *Capsicum chinense* (PI 209028, PI 224443, PI 238047, PI 238051, PI 257136, PI 439464, PI 594139, PI 485593, PI 439420, PI 281443); and one *Capsicum frutescens* (PI 631144). These selected genotypes represented cultivars originally acquired from world-wide different locations. Randomly selected fruits of ten plants of each genotype were harvested at full maturity on August 20, September 24, and October 15, 2015. In each instance, fruits were harvested from throughout the plants to reduce the effect of fruit position on the concentration of the compounds analyzed.

Extraction and quantification of capsaicinoids

At fruit maturity, fruit calyxes were removed and the fruits were chopped and processed for capsaicinoids extraction. Capsaicinoids were extracted by blending 50 g of homogenate (n=3) of fresh fruits with 100 mL of methanol for one min. The solvent extracts were decanted through 55 mm Whatman 934-AH glass microfiber filter discs (Fisher Scientific, Pittsburg, PA) and concentrated in a rotary vacuum evaporator (Buchi Rotovapor, Model 461, Flawil, Switzerland) at 35°C, chased with nitrogen gas (N_2), and reconstituted in 10 mL of methanol. A portion of each extract was subsequently passed through a 0.45 µm GD/X disposable syringe filter (Fisher Scientific, Pittsburg, PA). One µL (n=3) of this filtrate was injected into a gas chromatograph (GC) equipped with a nitrogen-phosphorus detector (NPD). GC separations were accomplished using a 25 m × 0.20 mm ID capillary column with 0.33 µm film thickness (HP-1). Operating conditions were 230, 250, and 280°C for injector, oven, and detector, respectively with a carrier gas (He) flow rate of 5.2 mL min^{-1}. Peak areas were determined using a Hewlett-Packard (HP) model 3396 series II integrator. Quantifications were based on average peak areas of 1 µL injections obtained from external purified standard solutions of capsaicin (N-vanillyl-8-methyl-6-nonenamide) and dihydrocapsaicin (Figure 2) obtained Sigma-Aldrich Inc. (Saint Louis, MO, USA) prepared in methanol. Under these GC/NPD conditions, retention times (RT values) were 9.1, 11.5, 11.8 min, for nordihydrocapsaicin, capsaicin, and dihydrocapsaicin, respectively. Peak identities were confirmed by consistent retention time and co-elution with standards under the conditions described above.

A gas chromatograph HP model 5890A equipped with a mass selective detector (GC/MSD) operated in total ion monitoring with electron impact ionization (EI) mode and 70 eV (electron volt) was also used for identification and confirmation of individual peaks. Standard solutions were used to prepare calibration curves (Figure 3). Mass spectrometric analysis of fruit extracts revealed fragments with identical molecular ions at m/z 305, m/z 307, and m/z 293, in addition to other characteristic fragment ion peaks that were consistent with the assignment of the molecular formulae of capsaicin ($C_{18}H_{27}NO_3$), dihydrocapsaicin ($C_{18}H_{29}NO_3$), and nordihydrocapsaicin ($C_{17}H_{27}NO_3$), respectively (Figure 4). All three compounds had a common benzyl cation fragment ($C_8H_9O_2$) at m/z 137 that was observed in all pepper extracts (Figure 5). The retention time and mass spectra of capsaicinoids isolated from the fruits of *Capsicum spp.* in this study matched

Figure 1 Pepper plants grown at the University of Kentucky South Farm, Fayette County, Lexington, KY (upper photo) and pepper fruits of *Capsicum* spp. selected in this study.

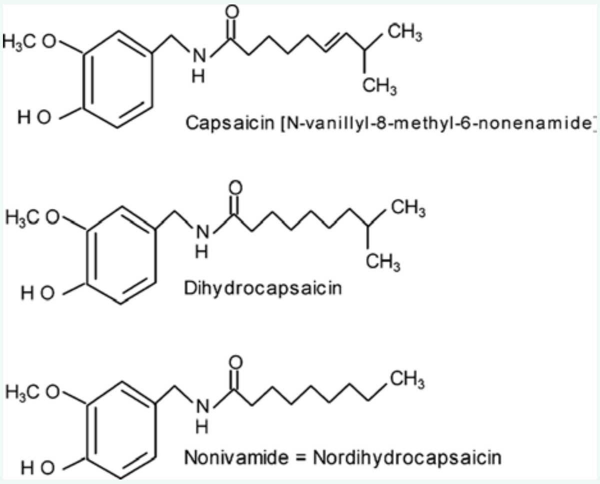

Figure 2 Chemical structures of capsaicin ($C_{18}H_{27}NO_3$, molar mass 305.42 g mol^{-1}; upper graph), dihydrocapsaicin ($C_{18}H_{29}NO_3$, molar mass 307.43 g mol^{-1}; middle graph), and nordihydrocapsaicin ($C_{17}H_{27}NO_3$, molecular mass 293.41 g mol^{-1}; lower graph).

those of their standards. Capsaicin and dihydrocapsaicin were the predominant capsaicinoids in the crude fruit extracts, although concentrations of each varied among genotypes tested. Nordihydrocapsaicin was always present at a very low concentration when compared to capsaicin and dihydrocapsaicin. Concentrations of nordihydrocapsaicin in fruits averaged 0.1 μg g^{-1} fresh fruit. Because of this low concentration, no further efforts were made to quantify nordihydrocapsaicin in fruit

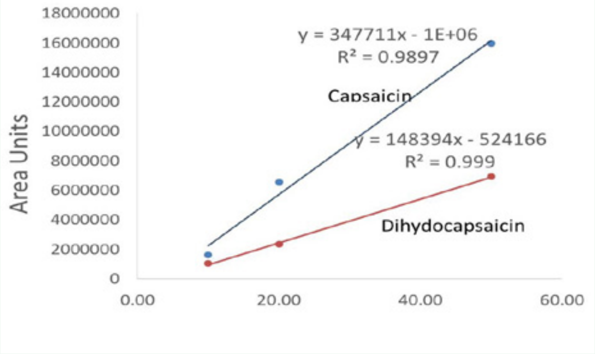

Figure 3 Standard curves of capsaicin and dihydrocapsaicin determined using a gas chromatograph equipped with a nitrogen phosphorus detector (GC/NPD).

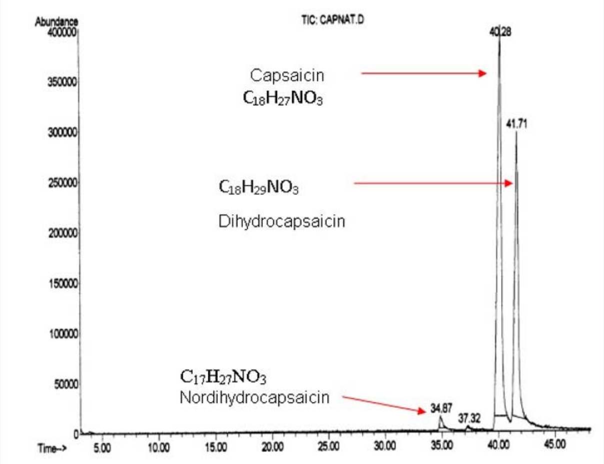

Figure 4 Gas chromatographic separation of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin determined using a gas chromatograph equipped with mass selective detector (GC/MSD).

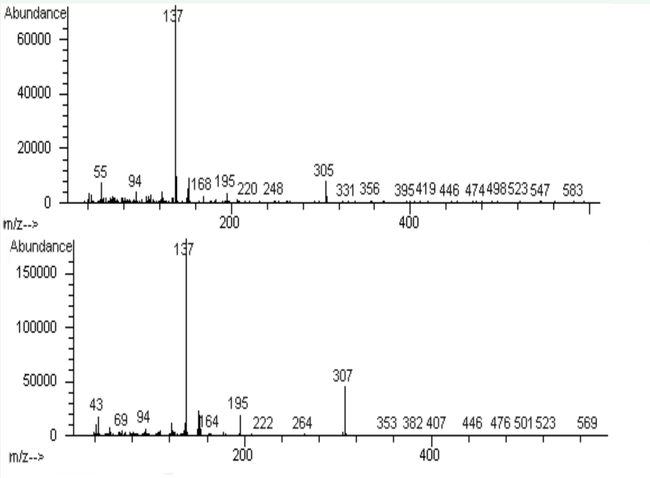

Figure 5 Ion fragments of capsaicin (upper graph) and dihydrocapsaicin (lower graph) determined using a gas chromatograph equipped with mass selective detector (GC/MSD). Note that the two molecular ions shared a benzyl cation fragment at m/z 137.

extracts. To determine the recovery of the extraction, cleanup, and quantification procedure, concentrations of capsaicin and dihydrocapsaicin in the range of 10–50 µg g^{-1} fresh fruit were added to 20 g of nonpungent bell pepper (*C. annuum*) fruits. Linearity over the range of concentrations was determined using regression analysis. Concentrations of the two dominant capsaicinoids, capsaicin and dihydrocapsaicin, as well as total capsaicinoids (capsaicin plus dihydrocapsaicin) were statistically analyzed using ANOVA. Means were compared using multiple range test (SAS Institute Inc.). [22]

RESULTS AND DISCUSSION

Analysis of pepper fruit extracts for capsaicin and dihydrocapsaicin indicated that concentrations and relative proportions of their capsaicinoids content varied significantly among genotypes of the four species tested. PI 631144 of *C. frutescens* contained the greatest concentrations of capsaicin compared to other genotypes tested. Concentrations of capsaicin ranged from 0.6 and 323 µg g^{-1} fresh fruits in PI 169129 and PI 631144, respectively (Figure 6).

Concentration of dihydrocapsaicin also varied among genotypes and was lowest in PI 169129 and greatest in PI 123474 (Figure 7). In most cases, capsaicin concentrations were greater than dihydrocapsaicin as shown in the chromatogram (Figure 4). Concentration of total capsaicinoids (capsaicin and dihydrocapsaicin) was greatest (465 µg g^{-1} fresh fruits) in PI 631144 and lowest in (1.2 µg g^{-1} fresh fruits) in PI 169129 compared to all genotypes analyzed (Figure 8).

The overall average of the three pepper harvests revealed that among the field-grown genotypes, PI-631144 produced the greatest concentration of total capsaicinoids (capsaicin plus dihydrocapsaicin).

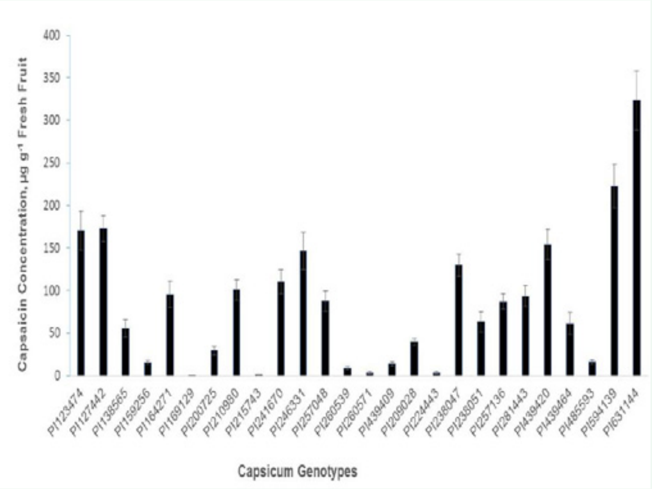

Figure 6 Concentrations of capsaicin in pepper fruits of twenty-six genotypes of Capsicum spp. grown at the University of Kentucky Research Farm, Fayette County, KY. Each value is an average of three replicates ± standard error, where no bar is shown, it is less than the size of the symbol.

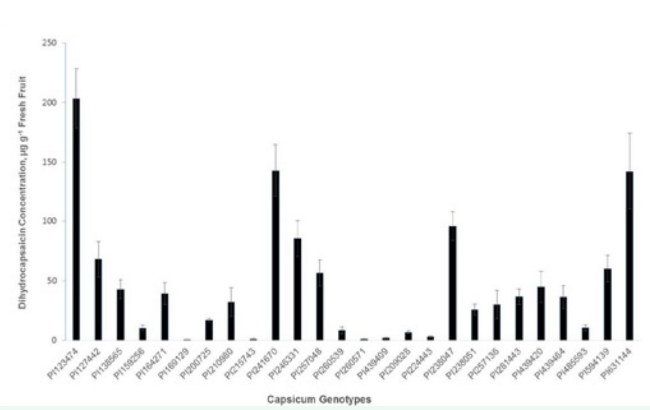

Figure 7 Concentrations of dihydrocapsaicin in pepper fruits of twenty-six genotypes of *Capsicum* spp. grown at the University of Kentucky Research Farm, Fayette County, KY. Each value is an average of three replicates ± standard error, where no bar is shown, it is less than the size of the symbol.

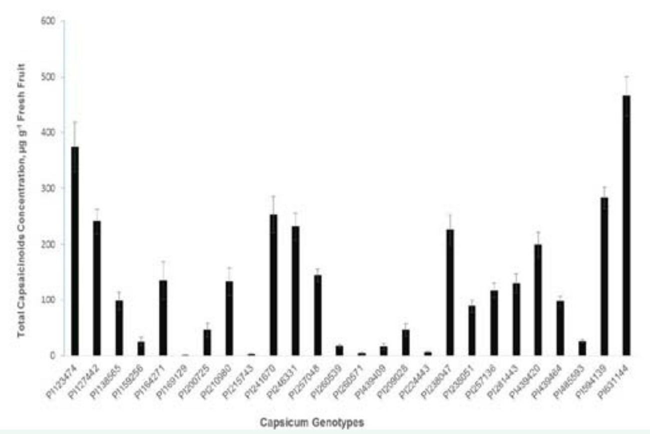

Figure 8 Concentrations of total capsaicin and dihydrocapsaicin in pepper fruits of genotypes of *Capsicum* spp. grown at the University of Kentucky Research Farm, Fayette County, KY. Each value is an average of three replicates ± standard error, where no bar is shown, it is less than the size of the symbol.

The results of this investigation revealed that plants of PI 169129 (Figure 9, left photo) of *C. annuum* averaged 49 cm tall and 49.5 cm in diameter. PI 631144 plants (Figure 9, right photo) of *C. frutescens* averaged 56 cm tall and 57 cm in diameter. According to the results in Table (1), the average fruit weight and length of PI 169129 (12.6 and 10.3 cm, respectively) were significantly greater ($P < 0.05$) than PI 631144 (8.5 and 7 cm, respectively). Whereas, fruit diameter and fruit wall thickness were significantly similar. Fruits were harvested three times during the growing season on August 20, September 24, and October 15, 2015. At each harvest, fruits were weighed. There was a significant increase in total fruit yield of PI 169129 plants (3.2 kg $plant^{-1}$ compared to PI 631144 plants (1.07 kg $plant^{-1}$) indicating that PI 169129 satisfies growers' need of high yield, whereas PI 631144 satisfies consumer needs of healthy food (great concentrations of capsaicinoids that have anticancer and other antioxidants properties).

Figure 9 Morphological structures of PI 169129 (left photo) and PI 631144 (right photo) of *Capsicum* plants grown at the University of Kentucky South Research Farm, Fayette County, KY.

Table 1: Fruit quality characteristics and yield of two genotypes of *Capsicum* spp. grown at the University of Kentucky South Farm (Fayette County, KY).

Pepper Species	Genotype	Fruit Length, cm	Fruit Width, cm	Fruit Weight, g	Fruit Wall thickness, mm	Weight of Fruits Plant, $^{-1}$ g
Capsicum annuum	PI 169129	10.32 ± 1.65 a	2.09 ± 0.32 a	12.60 ± 2.55 a	1.32 ± 0.32 a	Total yield Plant $^{-1}$ = 3197 ± 422 a
Capsicum frutescens	PI 631144	6.97 ± 0.84 b	2.22 ± 0.11 a	8.45 ± 1.34 b	1.76 ± 0.53 a	Total yield Plant $^{-1}$ =1073 ± 106 b

Each value in the table is an average of ten replicates ± std. error. Statistical analyses were carried out among fruits of two genotype for each characteristic. Values in each column for each characteristic having the same letter are not significantly different (*P*> 0.05, SAS Institute Inc.) [22].

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