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Pungency in *Capsicum chinense*: Variation among countries of origin

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Fruits of 63 accessions of *Capsicum chinense* Jacq. from the USDA/ARS Capsicum germplasm collection were analyzed for two major capsaicinoids, capsaicin and dihydrocapsaicin, using gas chromatography with nitrogen phosphorus detection (GC/NPD). The objectives of the present investigation were: (i) to quantify the major capsaicinoids (capsaicin and dihydrocapsaicin) in fruits of *Capsicum chinense* accessions and (ii) to identify accessions containing great concentrations of capsaicinoids among countries of hot pepper origin. Seeds of *C. chinense* accessions received from Belize, Brazil, Colombia, Ecuador, Mexico, Peru, Puerto Rico, and United States were field grown in a silty-loam soil. Mature fruits were analyzed for major capsaicinoids content. Capsaicin concentrations were generally greater than dihydrocapsaicin. Fruits of *C. chinense* accession PI640900 (USA) contained the greatest concentration of capsaicin (1.52 mg g⁻¹ fruit) and dihydrocapsaicin (1.16 mg g⁻¹ fruit), while total major capsaicinoids in the fruits of PI438648 (Mexico) averaged 2 mg g⁻¹ fruit. These two accessions were identified as potential candidates for mass production of major capsaicinoids that have health-promoting properties and for use as a source of pest control agents in agricultural fields.

**Keywords:** Hot peppers; phytochemicals; capsaicin; dihydrocapsaicin; gas chromatography with nitrogen phosphorus detection (GC-NPD).

**Introduction**

The USDA *Capsicum* germplasm collection contains many thousands of accessions of *Capsicum chinense*, although only limited information is currently available on the capsaicinoids content of the fruits of these accessions. 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.). Varieties of these *Capsicum* spp. exhibit varying degrees of pungency that reflect the relative concentrations of capsaicin [N-vanillyl-8-methyl-6-nonenamide], dihydrocapsaicin, and other analogs¹⁻² that are known collectively as capsaicinoids.³ Capsaicin is the most pungent member in this group. All capsaicinoids exhibit antioxidant activity and antimitagenic and anticarcinogenic properties.⁴ Capsaicin and dihydrocapsaicin typically account for an estimated 80-95% of the naturally occurring capsaicinoids in peppers.⁵⁻⁶ Other forms, i.e. nordihyrocapsaicin, homocapsaicin, and homodihydrocapsaicin are generally present in trace amounts.

Pungency is a key characteristic associated with members of the genus *Capsicum* and is also an important fruit quality attribute.⁷ Pungent chili varieties are grown for their food value, health-promoting properties,⁸ and also as a source of capsaicinoids that have a variety of medicinal uses.⁹ There is a growing interest in the enhancement of compounds in foods having health-promoting attributes such as capsaicinoids and their antioxidant properties.¹⁰⁻¹¹ Pungency associated with many forms makes the fresh or dried fruit a desirable spice and a valuable international commodity.¹² At the present time, 90 percent of U.S. chili pepper production occurs in New Mexico, eastern Arizona, and western Texas.¹³ *Capsicum chinense* has been referred to as the most cultivated pepper in South America.¹² 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 *Capsicum*...
chinense. In contrast, capsaicinoids may be entirely absent in Bell-type peppers (*C. annuum*). In fact, the concentrations of individual capsaicinoids and the proportion of capsaicin/dihydrocapsaicin fluctuates within and among species. In addition, absolute capsaicinoid concentrations are subject to a variety of environmental, cultural, and other factors.

The literature review has shown that extracts or powders from the fruit of pungent pepper varieties possess insecticidal activity. Hot pepper (*Capsicum* spp.) was superior to other plant extracts in protecting bean (*Phaseolus vulgaris*) plants from various insect pests including the foliar beetle, *Ootheca bennigseni*, and larvae of pod borers, *Maruca testulalis* and *Heliothis armigera*. Hot pepper extracts were found as effective as lindane (a synthetic organochlorine insecticide) in protecting bean plants from insect pests. Cowles et al. reported that chili pepper powder deterred oviposition of the onion fly, *Delia antiqua*. Capsaicin in hot pepper has been reported to reduce larval growth of the spiny bollworm, *Earias insulana*. The use of oleoresin from *Capsicum* has been reported effective as a repellent against cotton pests. Capsaicin can provide better control of cabbage worms than Karate (*λ*-cyhalothrin), a synthetic insecticide.

The literature contains little information on variability in capsaicinoid content among varieties of *C. chinense* and among their countries of origin. This study was undertaken to examine accessions of hot pepper fruits of *C. chinense* in the U.S. Department of Agriculture (USDA) *Capsicum* germplasm collection for variability in fruit concentrations of capsaicinoids that might subsequently be utilized to enhance fruit content of capsaicinoids. The main objectives of this investigation were: 1) to conduct a survey to quantify the major capsaicinoids (capsaicin and dihydrocapsaicin) in fruits of *Capsicum chinense* from various countries of origin (Belize, Brazil, Colombia, Ecuador, Mexico, Peru, Puerto Rico, and United States), and 2) to identify the accessions within these that contain the greatest concentrations of capsaicin and dihydrocapsaicin.

### Materials and methods

Seeds of a total of 63 accessions of hot pepper identified in the genebank inventory as Plant Introduction or Griffin (PI or GRIF) of *Capsicum chinense* accessions were obtained from the USDA/Agricultural Research Service (ARS) genebank in Griffin, GA and sown in the greenhouse in Woodland, CA. The accessions of *C. chinense* represented germplasm originally acquired from a variety of locations including: Belize (n = 9), Brazil (n = 7), Colombia (n = 8), Ecuador (n = 6), Mexico (n = 10), Peru (n = 10), Puerto Rico (n = 6), and United States (n = 7). The taxonomic identification of these accessions was confirmed by USDA/ARS, Griffin, GA. Seedlings were transplanted in the field in May 2006 into rows about 1.5 m apart and 0.25 m between plants within rows. Plants were fertilized and weeded as needed. Randomly selected fruits of each accession were harvested at full maturity. Fruit calyxes were removed and the fruits were chopped and shipped overnight to Kentucky State University, Environmental Toxicology Laboratory, Frankfort, KY where they were processed for capsaicinoid analyses. In each instance, fruits were harvested from throughout the plants to reduce the effect of fruit position on the concentration of the compounds analyzed.

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 (N₂), and constituted in 10 mL of methanol. A portion of each extract was subsequently passed through a 0.45 μm graded density (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 internal diameter (ID) capillary column with 0.33 μm film thickness (HP-1). Operating conditions were 230°C, 250°C, 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 standard solutions of capsaicinoids prepared in methanol. Under these conditions, retention times (Rₜ) were 11.50 and 11.75 min for capsaicin and dihydrocapsaicin, respectively. Peak identities were confirmed by consistent retention time and coelution with standards under the conditions described above. A HP gas chromatograph (GC) model 5890A equipped with a mass spectrometer (GC/MS) operated in total ion monitoring with electron impact ionization (EI) mode and 70 eV electron energy was also used for identification and confirmation of individual peaks. The instrument was auto-tuned with perfluorotributylamine (PFTBA) at m/z 69, 210, and 502. Purified standards of capsaicin (N-vanillyl-8-methyl-6-noneamide) and dihydrocapsaicin were obtained from Sigma-Aldrich Inc. (Saint Louis, MO 63103, USA) and used to obtain calibration curves. To determine the recovery of the extraction, clean-up, and quantification procedure, concentrations of capsaicin and dihydrocapsaicin in the range of 20–200 μg g⁻¹ fruit were added to 20 g of bell pepper (*C. annuum*) fruits. Recoveries of the added capsaicin and dihydrocapsaicin were 98% and 95%, respectively.

Linearity over the range of concentrations was determined using regression analysis. Concentrations of the two
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Results and discussion

Analysis of capsaicin and dihydrocapsaicin in 63 genebank accessions of C. chinense indicated that the concentrations and relative proportions of these two capsaicinoids varied significantly between accessions from the same country of origin, and between countries of origin. Eleven accessions out of the 63 accessions analyzed contained the greatest concentrations of total capsaicinoids (Figure 1) compared to other accessions tested. Concentrations of capsaicin, dihydrocapsaicin, and total capsaicinoids in the remaining 52 accessions are presented in Figure 2 A–C. Concentrations of capsaicin varied among accessions from 1.52 mg g\(^{-1}\) fruit in PI640900 (Figure 1) to 0.01 mg g\(^{-1}\) fruit in PI257050 (Figure 2 A). Concentrations of dihydrocapsaicin varied among accessions from 1.16 mg g\(^{-1}\) in PI640900 to 0.003 mg g\(^{-1}\) in PI257050. Concentrations of total capsaicinoids (capsaicin and dihydrocapsaicin) varied among accessions from 2.68 mg g\(^{-1}\) in PI640900 to 0.01 mg g\(^{-1}\) in PI-257050. Accession PI640900 contained the greatest concentrations of each of the two pungent compounds of hot pepper among all the accessions tested.

Nordihydrocapsaicin was always present at very low concentrations when compared to capsaicin and dihydrocapsaicin. Concentrations of nordihydrocapsaicin in fruits of C. chinense averaged 0.26 µg g\(^{-1}\) fresh fruit. Because of these low concentrations, no further effort was made to quantify nordihydrocapsaicin in the fruit extracts.

In most cases, capsaicin concentrations were greater than dihydrocapsaicin, and total capsaicinoids content (capsaicin plus dihydrocapsaicin) varied from 1.36 mg g\(^{-1}\) fruit (Mexico) to 0.2 mg g\(^{-1}\) fruit (Puerto Rico). Figure 3 illustrates the variability of total capsaicinoid concentrations among the eight countries of origin included in this survey. Overall statistical analysis revealed that greatest concentrations of total capsaicinoids were detected in accessions collected from Mexico, the United States, and Brazil. Variability in the concentrations of major capsaicinoids among accessions of C. chinense and among countries of origin may enable the identification and/or development of germplasm with high levels of health-promoting capsaicinoids. In addition, this information on fruit concentration

![Graph](image-url)

Fig. 1. Concentrations of capsaicin (upper graph), dihydrocapsaicin (middle graph), and total capsaicinoids (capsaicin and dihydrocapsaicin) (lower graph) in 11 accessions of Capsicum chinense grown from seeds originated from different countries of origin (BR = Brazil, CO = Columbia, ME = Mexico, US = United States) having greatest concentrations of capsaicinoids among 63 accessions. Bars accompanied by different letter(s) indicate significant differences (P < 0.05) using Duncan’s multiple range test.
Fig. 2. Concentrations of capsaicin (Figure 2-A), dihydrocapsaicin (Figure 2-B), and total capsaicinoids (capsaicin plus dihydrocapsaicin) (Figure 2-C) in 52 accessions of *Capsicum chinense* grown from seeds originated from different countries of origin. Vertical bars indicate ± standard error; where no bar is shown, it is less than the size of the symbol.
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Fig. 3. Concentrations of capsaicin (upper graph), dihydrocapsaicin (middle graph), and total capsaicinoids (capsaicin and dihydrocapsaicin) (lower graph) in fruits of *Capsicum chinense* grown from seeds collected from 8 countries (ME = Mexico, US = United States, BR = Brazil, CO = Columbia, EC = Ecuador, PE = Peru, BE = Belize, PR = Puerto Rico). Bars accompanied by different letter(s) indicate significant differences ($P < 0.05$) using Duncan’s multiple range test.

of capsaicinoids of *C. chinense* with elevated levels of health-promoting compounds has increased potential for use as pest control agents.

Out of the 63 accessions tested, accessions PI 438648 (Mexico) and PI 640900 (USA) were identified as potential candidates for mass production of major capsaicinoids among accessions of *Capsicum chinense*.

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