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

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The main objective of this investigation was to evaluate fruits of *C. chinense* accessions for their concentration of β-carotene, ascorbic acid, and phenols for use as parents in breeding for these phytochemicals. Mature fruits of 63 accessions of *C. chinense* originally acquired from Belize, Brazil, Colombia, Ecuador, Mexico, Peru, Puerto Rico, and the United States were analyzed for their chemical composition. Fruits of *C. chinense* accessions PI-152452 (Brazil) and PI-360726 (Ecuador) contained the greatest concentrations of ascorbic acid (1.2 and 1.1 mg g⁻¹ fresh fruit, respectively), while PI-438648 (Mexico) contained the greatest concentration of total phenols content (349 µg g⁻¹ fresh fruit) among the other 63 accessions tested. Accession PI-355817 from Ecuador contained the greatest concentrations of β-carotene (8 mg g⁻¹ fresh fruit). These accessions were identified as potential candidates for mass production of antioxidants with health-promoting properties.

Keywords: Phytochemicals; β-carotene; ascorbic acid; capsaicin; total phenols.

Introduction

There is a growing interest in the enhancement of compounds in food that possess health-promoting attributes such as antioxidant properties, which were previously regarded as non-nutritive.[1] Non-nutritive bioactive compounds are referred to as “phytochemicals.” Compounds capable of acting as antioxidants are likely to be beneficial by augmenting cellular defenses and by acting as free radical scavengers and reducing agents.[2] The fruits of green or red pepper (*Capsicum* spp.) contain numerous compounds with antioxidant activity including β-carotene, various alkaloids, phenols, and ascorbic acid.[3–5] (Fig. 1). The hydrocarbon carotenoid β-carotene is found widely in chloroplasts of higher plants. Beta-carotene possesses provitamin-A activity and is a powerful antioxidant.[6] The cancer-preventive activities of carotenoids have been associated with their antioxidant properties.[7]

Carotenoids are synthesized in plants, fungi, bacteria, and algae, but not humans. Perera and Yen[8] reported that consumption of carotenoid-rich foods reduced the incidence of several diseases such as cancers, cardiovascular diseases, age-related macular degeneration, cataracts, diseases related to low immune functions, and other degenerative diseases.

Capsaicinoids are alkaloids important for pharmaceutical industry for their neurological effects. In the fruit of *Capsicum*, they account for pungency or 'heat’. When used at low levels in the diet, capsaicinoids significantly decrease serum, myocardial and aortic total cholesterol levels.[9] The two major capsaicinoids capsaicin and dihydrocapsaicin are responsible for about 90% of the pungency in hot pepper.

Plant phenols may interfere with stages of the cancer process, potentially resulting in a reduction of cancer risk. They prevent oxidative damage to biomolecules such as DNA, lipids and proteins which play a role in chronic diseases such as cancer and cardiovascular disease.[10] Plant phenols include simple phenols, flavonoids, anthocyanins, lignans and lignins, stilbenes and tannins. The role of phenols as antioxidants with properties similar to vitamins C, E, and β-carotene have prompted a number of studies of these compounds. A wide variety of phenolic compounds derived from spices, like capsaicin possesses
Ascorbic acid

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{CH}_3 \\
\text{CH}_3 & \quad \text{CH}_3
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{O} & \quad \text{COOH} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

β-carotene

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{CH}_3 \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{H} & \quad \text{O}
\end{align*}
\]

Chlorogenic acid

![Chemical structures](image)

**Fig. 1.** Chemical structures of ascorbic acid, β-carotene, and chlorogenic acid (a phenolic natural product).

Some mammalian metabolites of polyphenols and tannins (PPT) may protect the vascular endothelium. Diets rich in PPT may have the ability to protect against type-2 diabetes through their effects on glucose absorption and associated hormones.[12]

When derivatives of ascorbic acid were tested on cancer cells, ascorbic acid esters revealed promising anticancer activity.[13] In addition, ascorbic acid as found in most fruits and vegetables protects against heart disease, high cholesterol, high blood pressure, and cancer.[14] Some pepper varieties have significant inhibitory effects on carbohydrate-degrading enzymes such as intestinal α-glucosidases that are related to glucose absorption. Hydrolysis of dietary carbohydrates, such as starch, is the major source of glucose in the blood. It has been previously suggested that screening of pepper accessions for their ability to control/regulate intestinal glucose absorption may result in an alternative means for the dietary management of type-2 diabetes.[15]

*Capsicum chinense* has been referred to as the most cultivated pepper in South America.[16] Pepper varieties are grown for their food value, health-promoting properties,[17] and also as a source of capsaicinoids that have a variety of medicinal uses.[18] However, limited information is currently available on variability in the composition of the fruit of this species for health-promoting compounds. Variability in the presence and concentration(s) of phytochemicals in pepper fruit could be a factor affecting their selection for use as parents in an improvement program.

The objectives of this investigation were: 1) to determine the concentration of β-carotene, total phenols, and ascorbic acid in 63 hot pepper accessions of *Capsicum chinense* collected from eight countries and 2) to identify accessions within those with greatest concentrations of β-carotene, ascorbic acid, and total phenols for potential use in crop improvement.

**Materials and methods**

Seeds of 63 accessions of *Capsicum chinense* were obtained from U.S. Department of Agriculture Agriculture Research Service (USDA/ARS) *Capsicum* germplasm collection in Griffin, GA. These accessions represented cultivars and landraces 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). Seeds were sown in March and the seedlings were transplanted in the field in May 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 from 150 days old plants were harvested at full maturity.

At harvest, pepper fruits were cut into small pieces and 30 g representative subsamples were blended in a house-
Antioxidants in Capsicum chinense hold blender at high speed with 100 mL of acetone for 2 min in dim light to extract β-carotene. The homogenate was filtered with suction through a Buchner funnel containing Whatman filter paper No.1 (Fisher Scientific, Pittsburgh, PA). The resulting thick paste was extracted twice with acetone until the extract was colorless. The filtrates were combined, transferred to separatory funnel containing 50 mL of 4% aqueous NaCl and 100 mL of petroleum ether (BP 40–60°C). Absorption of the petroleum ether layer was measured at 450 nm in dim light. A calibration curve was prepared for each group of samples using 99% pure β-carotene in the range of 10–100 µg mL⁻¹.

Representative fruit samples (20 g) were blended with 150 mL of ethanol to extract phenols. Homogenates were filtered through Whatman No. 1 filter paper and one mL aliquots of filtrate were used for determination of total phenols using a standard calibration curve (1 to 16 µg mL⁻¹) of chlorogenic acid. Ascorbic acid was extracted by blending 20 g of fruit with 100 mL of 0.4% (w/v) oxalic acid solution and determined by the dichlorophenolindophenol method.

Purified standards of β-carotene, ascorbic acid, and chlorogenic acid were obtained from Sigma-Aldrich Inc (Saint Louis, MO 63103, USA) and used to obtain calibration curves. Concentrations of each compound, expressed on a fresh weight basis, were statistically analyzed using ANOVA procedure. Means were compared using Duncan’s multiple range test (SAS Institute, 2003).

Results and discussion

Concentrations of the analyzed phytochemicals varied significantly among accessions from the same country of origin, and between countries of origin. Concentrations of ascorbic acid in two accessions, PI 152452 (Brazil) and PI 360726 (Ecuador), were significantly higher (1,224 and 1,139 µg g⁻¹ fresh fruit, respectively) (Fig. 2, upper graph) than other accessions analyzed. These accessions may be useful as parents in hybridizations to produce high ascorbic acid containing varieties. Fruits of accessions that originated in Ecuador contained the greatest concentration of ascorbic acid (Fig. 2, lower graph), while PI 281424 from Peru contained the lowest (266 µg g⁻¹ fresh fruit) (Fig. 3). Among the 63 accessions analyzed, concentrations of total phenols were significantly higher in PI 438248, PI 159248, and PI 360900 (Fig. 4, upper graph). Seeds of these accession originated in Mexico and the US, respectively (Fig. 4, upper graph). Total phenols concentrations were generally low in fruits of accessions from Belize (Fig. 5).

Figure 6 illustrates the variability among 10 accessions with the greatest concentrations of β-carotene (Fig. 6, upper graph) and among the eight countries of origin included in this investigation. Statistical analyses reveal that greatest concentrations of β-carotene were found in fruits of accessions from Ecuador (Fig. 6, bottom and Fig. 7). Our previous work on capsaicinoids in C. chinense revealed pronounced variability in total capsaicinoids (capsaicin plus dihydrocapsaicin) concentrations among the 63 C. chinense
accessions. Fruits of accession PI 640900 (USA) contained the greatest concentration of capsaicin (1.5 mg g\(^{-1}\) fresh fruit) and dihydrocapsaicin (1.2 mg g\(^{-1}\) fresh fruit), while total major capsaicinoids in the fruits of PI 438648 (Mexico) averaged 2 mg g\(^{-1}\) fruit (data not shown).

The present investigation is a continuation of our work on *Capsicum chinense* of the world and suggests that great variability exists within *Capsicum chinense* for total phenols, ascorbic acid, and \(\beta\)-carotene. These compounds have antioxidant properties and are thus important quality attributes.

**Fig. 4.** Concentrations of phenols in 10 top accessions of *Capsicum chinense* (upper graph) grown from seeds of different countries of origin; Mexico (ME), United States (US), Ecuador (EC), Peru (PE), Brazil (BR), and Colombia (CO) having greatest concentrations of total phenols among 63 accessions tested and concentrations of phenols among hot pepper countries of origin (lower graph). Bars accompanied by different letter(s) indicate significant differences (\(P < 0.05\)) using Duncan’s multiple range test (SAS Institute, 2003).

**Fig. 5.** Concentrations of total phenols in 53 accessions of *Capsicum chinense* grown from seeds of different countries of origin; Belize (BE), Brazil (BR), Colombia (CO), Ecuador (EC), Mexico (ME), Peru (PE), Puerto Rico (PR), and the United States (US). Vertical bars indicate ± standard error.
Fig. 6. Concentrations of β-carotene in 10 top accessions of *(Capsicum chinense)* (upper graph) grown from seeds of different countries of origin; Ecuador (EC), United States (US), Puerto Rico (PR), Brazil (BR), and Peru (PE) having greatest concentrations of β-carotene among 63 accessions tested and concentrations of β-carotene among hot pepper countries of origin (lower graph). Bars accompanied by different letter(s) indicate significant differences (*P* < 0.05) using Duncan’s multiple range test (SAS Institute, 2003).

Fig. 7. Concentrations of β-carotene in 53 accessions of *(Capsicum chinense)* grown from seeds of different countries of origin; Belize (BE), Brazil (BR), Colombia (CO), Ecuador (EC), Mexico (ME), Peru (PE), Puerto Rico (PR), and the United States (US). Vertical bars indicate ± standard error.
Variability for these traits might be utilized via plant breeding approaches to produce fruit desirable to the consumer for their value-added health-promoting characteristics.

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