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Capsaicinoids in Vegetative Organs of *Capsicum annuum* L. in Relation to Fruiting

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Pepper (cv. Padrón) shows a spatial gradient in the content of phenolic compounds, and particularly of capsaicinoids, along the stem. These compounds were consistently more abundant in apical fruits than in fruits belonging to middle and basal segments. Analysis of the two principal capsaicinoids in fruits showed that the proportion of capsaicin was always higher than that of dihydrocapsaicin. Capsaicinoids were also found to be present in vegetative organs, such as stem and leaves. In this case, the proportion of individual capsaicinoids was different than that in fruits, and dihydrocapsaicin was found to be more abundant. To find out whether the capsaicinoids in vegetative organs came from the fruits, the floral buds were removed and fruit formation was prevented. Capsaicinoids were not detected in the stem and leaves of floral bud-deprived plants, suggesting that they did originate from the fruit.

KEYWORDS: **Capsaicinoids; *Capsicum annuum*; vegetative organs; fruits**

INTRODUCTION

Phenolic compounds are present in all plant tissues and are often the most abundant secondary metabolites in fruits. These compounds, whose levels vary strongly during growth and maturation, are also important because of their contribution to the sensory quality of the fruits: color, astringency, bitterness, and flavor (*1*).

There is a group of phenolic compounds characteristic of some fruits of the genus *Capsicum*, capsaicin and other related compounds, which are commonly called capsaicinoids (*2*). Capsaicin and dihydrocapsaicin are the most abundant pungent principles in hot peppers (*3*), and are responsible for 90% of the total pungency (*4*). They both are the main products of a metabolic pathway which has been well characterized. Capsaicinoids are synthesized by the condensation of vanillylamine with a short-chain branched fatty acid. The fatty acid moiety is biosynthetically derived from valine, whereas the vanillylamine moiety comes from L-phenylalanine, via the phenylpropanoid pathway (*5*).

The accumulation of capsaicinoids is primarily associated with a particular developmental stage of a single organ of the plant: the fruit. Many studies have reported on the accumulation of capsaicinoids in *Capsicum* fruits in relation to the fruit age, size, and stage of development (*3*, *6*−*10*). In all the cases, the capsaicinoids begin to accumulate in the early stages of fruit development and are accumulated at a maximum rate as the fruit approaches the end of the growth phase. This accumulation continues to increase after the length of the fruit reaches a maximum value (*11*). However, the level of capsaicinoids varies according to the different pepper cultivars examined (*3*, *6*−*10*).

The site of capsaicinoid accumulation in pepper fruits was confirmed by Iwai et al. (*3*) by tracer experiments. They observed that the radioactivity of capsaicinoid is much higher in the placenta than in the pericarp at any stage. Later, members of the same group (*12*), examined the cellular structure of the placenta using a light microscope, and observed that some morphological changes took place in the epidermal tissue of the placenta during maturation. The epidermal cells of the placenta appeared to be the site of capsaicinoid accumulation. The small amount of capsaicinoid detected in the pericarp and seeds, however, might be due to the adherence of a small quantity of capsaicinoid coming from the placenta. In their study on *C. annuum* L. cv. Jalapeño, Ishikawa et al. (*13*) observed that capsaicinoids were accumulated in the placenta, and they also found capsaicinoids to be present in other tissues of the plant, but in much smaller quantities than those in the placenta.

Other studies on capsaicinoids have demonstrated that capsaicinoid content is genetically controlled, but also subjected to environmental variables such as temperature, light, soil moisture, and fertilization level (*14*−*16*). Recently, Harvell and Bosland (*17*) have also related the capsaicinoid content of chile genotypes to different environments, and the pungency of the chile fruit to the node position (*18*).

Given the fact that, despite the numerous studies on capsaicinoids and their accumulation in the fruit, very little information has been put forth on the presence of these compounds in the vegetative organs of the plant, the aim of this study was to

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analyze the capsaicinoid content in these organs, and to relate it to variation of these metabolites in the fruits of different regions of the plant.

MATERIALS AND METHODS

Plant Material. *Capsicum annuum* L. cv. Padrón was grown in a greenhouse on the campus of the University of La Coruña from April to September, for two consecutive years. Anthesis began about 3 months after germination; individual flowers were numbered, and the dates of flowering were recorded. Pepper fruits were classified into five different stages from 14 (stage 1) to 42 (stage 5) days after flowering (*10*). To analyze possible changes of secondary metabolites in different regions of the plant, the total plant height was divided into three segments: bottom, middle, and top. The different organs (fruits, leaves, and stems) were harvested at the same time in the three segments and analyzed separately.

Extraction and Quantification of Capsaicinoids by HPLC. Capsaicinoids were extracted from Padrón pepper fruits using the technique described by Collins et al. (*19*) with modifications (*10*).

Pepper fruits were oven-dried at 60 °C for 2−5 days; they were then ground using a laboratory mill and stored in sealed plastic tubes at room temperature prior to extraction. The capsaicinoids were extracted from 1.0 g of the ground pepper in 10 mL of acetonitrile by heating at 80 °C for 4 h. The suspended material was allowed to settle, and the supernatant was extracted and centrifuged at 100*g* for 10 min. Next, it was filtered (0.45-μm Whatman filter on a 10-mL disposable syringe) into a 2-mL glass sample vial, then capped and stored at 5 °C until analyzed. A 10-μL aliquot was used for each HPLC injection.

The samples were analyzed using a Waters LC616 System equipped with a Waters 717 plus autosampler, a Waters temperature control module, a Waters 996 photodiode array detector, and Millennium software for data processing. Reversed-phase HPLC was carried out on a Spherisorb ODS2 C_{18} column (5 μm particle size, 150 mm × 46 mm). A precolumn guard cartridge Spherisorb ODS2 C_{18} column was also used. The capsaicinoids were determined under the following HPLC operating conditions: 25 °C, a flow rate of 1 mL min^{-1}, and a 14 min run. The mobile phase was isocratic, with 50% solvent A (100% acetonitrile, HPLC grade) and 50% B (10% acetonitrile, HPLC grade).

Standards of capsaicin (approximately 98%) and dihydrocapsaicin (approximately 90%) were obtained from Sigma Chemical Co. (St. Louis, MO) and were used for retention time verification and instrument calibration. The mean retention times of capsaicin and dihydrocapsaicin under these conditions were 7.36 and 10.38 min, respectively. These two capsaicinoids were identified and quantified in HPLC chromatograms both by co-injection and by comparing the retention times, absortion spectra, and areas of different peaks with those of an external standard.

Extraction and Determination of Soluble Phenolics. Soluble phenolics from Padrón pepper fruits were extracted by the same procedure as capsaicinoids. Total soluble phenols were determined using the Folin−Ciocalteu reagent according to the method of Singlenton and Rossi (*20*). The content of the soluble phenols was calculated from a standard curve obtained by using different concentrations of ferulic acid.

RESULTS AND DISCUSSION

Soluble Phenolics and Capsaicinoids in Fruits All Along the Plant. Fruits at stage 5 were analyzed for their phenolic compounds and capsaicinoid content, in the different segments (bottom, middle, and top) of the pepper plant (**Figure 1**). Phenolic compounds increased from 2.48 mg in the basal fruits to 3.21 mg in apical fruits. The pattern of capsaicinoid accumulation along the plant is the same as that of phenolics: the lowest content is found in the basal fruits (0.78 mg), and the highest is in the apical fruits (1.09 mg). However, we did not observe any significant changes in the dry weight in the fruits of the three segments (data not shown). If we analyze the two principal capsaicinoids, capsaicin and dihydrocapsaicin, the same tendency can be seen. In the apical segment, the content of both capsaicinoids was higher than that in the basal segment (**Figure 1**). Capsaicin was always the major component, constituting an average of between 58.7 and 63.0% of the total capsaicinoids studied. In general, there were no major changes in the proportion of the two capsaicinoids with a capsaicin/dihydrocapsaicin ratio ranging from 1.43 to 1.69.

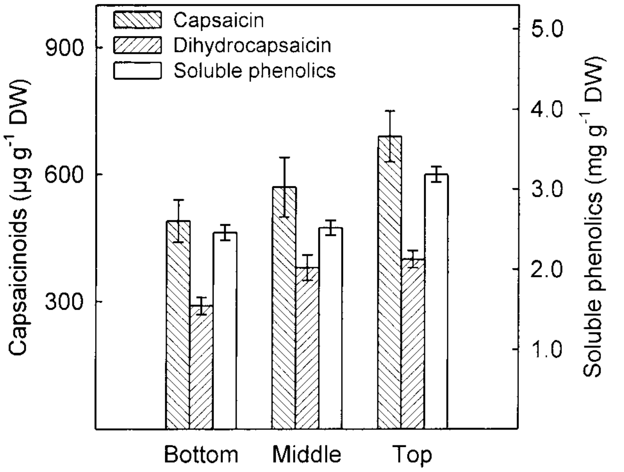

Figure 1. Soluble phenolics, capsaicin, and dihydrocapsaicin content in Padrón pepper fruits along the plant. Bars show SD.

The variation in pungency within the same cultivar can be observed when the plants are grown in different locations (*17*) or are subjected to different conditions, such as light and temperature (*15*). But the fruit's position on the plant also plays a important role in the accumulation of capsaicinoids, as well as in other aspects of the fruits, such as seed quality and their germination percentage (*21*). Zewdie and Bosland (*18*) measured the pungency of fruits from the different nodes of chile plants. They reported that the most pungent fruits came from the lower, or earliest nodes, on the plant, and speculated that the higher pungency was probably due to the fewer number of fruits on the plant, and that the early fruits received most of the nutrients responsible for capsaicin development. The later fruits had to share the nutrients, so they produced less capsaicin. In Padrón peppers, however, when the plants are in full yield, the nutrients are distributed among the fruits, and the content in capsaicinoids depends on environmental factors (*16, 22*). The higher capsaicinoid content in the apical zone can probably be attributed to the fact that these fruits receive a greater quantity of light than the fruits located in the middle and basal zones. In support of this theory, some authors have suggested that light exposure may be an important factor in capsaicinoid formation and accumulation (*3*).

Capsaicinoids in Vegetative Organs All Along the Plant. Two months after the analysis of fruits, capsaicinoids were analyzed in leaves and stems. They were found to be present in these vegetative organs. To determine whether there is a relation between fruiting and the presence of capsaicinoids in vegetative organs, we divided the plants into two groups. In the first group, the floral buds were removed as soon as they blossomed, thus preventing the development of fruits. The other group was allowed to develop fruits until maturation.

Both groups of plants, with and without fruits, presented very clear morphological differences from the beginning. Deflorated plants grew much faster than fruiting plants and, in some cases, differences in length were around 80 cm. Internodes of deflorated plants were longer, and apical leaves were more numerous and larger than those in fruiting plants.

Table 1. Percentage of Dry Matter in Leaves and Stems Collected at Different Zones of Padrón Peppers With and Without Fruits

	bottom		middle		top	
	deflorated	fruiting	deflorated	fruiting	deflorated	fruiting
leaves	13.52 ± 0.04	13.53 ± 0.01	15.21 ± 0.05	15.73 ± 0.01	17.80 ± 0.07	15.80 ± 0.06
stem	33.35 ± 0.04	26.37 ± 0.09	31.05 ± 0.06	24.80 ± 0.09	27.07 ± 0.08	23.88 ± 0.06

Table 2. Content[a] of Capsaicin and Dihydrocapsaicin in Leaves and Stems Collected at Different Zones of Padrón Pepper

	bottom		middle		top	
	CAP[b]	DHC[c]	CAP	DHC	CAP	DHP
leaves	1.04 ± 0.20	16.34 ± 0.40	3.03 ± 0.10	27.28 ± 0.30	19.99 ± 7.00	20.00 ± 3.50
stem	nd[d]	5.50 ± 0.70	0.75 ± 0.10	4.65 ± 0.30	7.00 ± 0.40	7.25 ± 1.00

[a] Data are expressed in μg/g DW ± SD. [b] CAP = capsaicin. [c] DHC = dihydrocapsaicin. [d] nd = not detected.

Table 1 shows changes in dry weight in vegetative organs of both plants, with and without fruits. Apical leaves have a greater dry weight content than those on the bottom. Moreover, there is no significant difference in leaves between plants without fruits and plants with fruits, except on the top. Unlike leaves, the stems have a higher percentage of dry matter on the bottom. In deflorated plants, the stems have a larger proportion of dry matter than those in fruiting plants. This difference is significant, ranging from 3% on the top to 7% on the bottom.

Differences in performance between fruiting and deflorated plants are probably due to the fact that fruits are used as a sink for assimilation during much of their growth cycle. Therefore, if plants are continuously deflorated, it is logical that partitioning of dry matter among organs would have a different balance. Hall (*23*) has shown similar results in another *C. annuum* cultivar, with the stem showing a significant increase in dry weight in deflorated plants with respect to those of fruiting plants.

In vegetative organs (leaves and stems) of plants where fruit development was prevented, we were unable to detect any capsaicinoid. This results contrast with that found in plants with fruits, where we observed the presence of capsaicinoids in vegetative organs, with the highest content in the apical segment and the lowest content in the basal segment (**Figure 2**). Capsaicinoid content was always higher in the leaves (**Figure 2A**) than in the stem (**Figure 2B**).

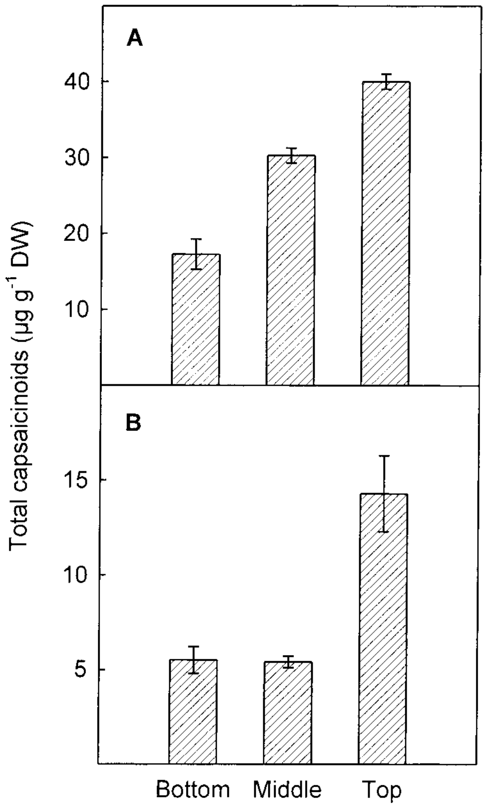

Figure 2. Capsaicinoids content in vegetative organs along Padrón pepper plants. Total capsaicinoids is the sum of capsaicin and dihydrocapsaicin content. A, leaves; B, stem. Bars show SD.

Ishikawa et al. (*13*), using Jalapeño peppers, reported that capsaicinoids were essentially accumulated in the placenta, although they did detect small quantities in other tissues, stems, and leaves. The content in vegetative tissues was only 0.7% of the amount found in the placenta. The Jalapeño cultivar is highly pungent, whereas the Padrón cultivar may be considered as mild, and therefore suitable for fresh consumption during the early stages. In Padrón peppers, the amount of capsaicinoids in the vegetative tissues is proportionally higher than that in the Jalapeño, with the capsaicinoid content in these organs related to the capsaicinoid content in the fruits of the same segments.

However, the most significant aspect that we found in the vegetative organs is the different proportion between capsaicin and dihydrocapsaicin, with dihydrocapsaicin being generally greater (**Table 2**). Also, the proportion of capsaicinoids changed throughout the plant. Thus, in basal leaves the content in dihydrocapsaicin is much higher than the content of capsaicin, but in the top the contents are the same. The stem showed similar proportions, but in lower amounts, and no capsaicin was detected in the bottom. These proportions differ greatly from those found in the fruits, where the major capsaicinoid always was capsaicin. Iwai et al. (*3*) speculated that interconversions among capsaicinoids, such as from dihydrocapsaicin to capsaicin, were unlikely to occur because the ratio of capsaicinoid composition did not change appreciably with the growth stage. But the same authors also observed that in fruits of the sweet pepper, after 10 days' post-harvest ripening under continuous light, there was a change in the proportion of capsaicinoids (*24*). Thus, dihydrocapsaicin, which is the vanillylamide of the saturated branched fatty acid, increased gradually, while capsaicin, which is the vanillylamide of its unsaturated counterpart, decreased. The continuous light probably had a stimulatory effect on some of the precursors, because the interconversion of the saturated fatty acid to unsaturated fatty acid must, therefore, take place at the free acid level. The vegetative organs, leaves and stems, analyzed in the Padrón pepper belonged to 8-month-old senescent plants whose fruits had an extremely disturbed capsaicinoid metabolism. The capsaicinoid content in these fruits was lower than that in the previous months (*25*) due to an active catabolism of capsaicinoids in the last stages. This catabolism may have affected capsaicin with a greater intensity than it did dihydrocapsaicin, and the latter could be exported largely to vegetative organs. Another possibility is that during the last stage, the capsaicinoids synthesized and exported by the old fruits were preferably dihydrocapsaicin, similar to what occurred during post-harvest ripening in fruits of the sweet pepper (*24*). Finally, it is also possible that the fruit does not export any capsaicinoid, but one or more signals (e.g., phytohormones) that trigger capsaicinoid biosynthesis in stem and leaves. In any case, fruiting is necessary for the accumulation of capsaicinoids in these vegetative organs.

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