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CAPSAICIN AND ASCORBIC ACID VARIABILITY IN CHILLI AND PAPRIKA CULTIVARS AS REVEALED BY HPLC ANALYSIS

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A B S T R A C T

Capsaicin and ascorbic acid contents of seven Indian peppers varieties/accessions from *Capsicum annuum* (CA 97, CCH, K1, KTPL19, Arka Abhir and Bayadagi Kaddi) and *C. frutescens* (CF1) species were determined using High Performance Liquid Chromatography (HPLC). Based on their pungency value, all the chilli accession/varieties (CA 97, CCH, K1, and CF 1) were classified as highly pungent peppers. The accession CF1 showed the highest concentration of capsaicin (445mg 100g $^{-1}$ DW) with corresponding pungency value of 71,200 SHUs and Arka Abhir variety showed the lowest capsaicin concentration (29mg 100g $^{-1}$ DW) with 4,672 SHUs of pungency value. Similarly, Bayadaggi kaddi variety showed the highest ascorbic acid content (189 mg/100 FW) and the accession CA 97 showed the lowest ascorbic acid contents (55.3 mg/100 FW). The variability in capsaicin and ascorbic acid content presented in the pepper germplasm can be exploited for breeding cultivars with improved nutritional qualities. Moreover, CF1and Bayadagi kaddi can be used as a potential source for capsaicin and vitamin C, respectively.

Keywords: *Capsicum annuum, Capsicum frutescens,* vitamin C, pungency.

Capsicum peppers are the most consumed vegetables or spices that are known for their pungency, pigment, and nutrition content. The pungency of peppers is attributed to the presence of a group of compounds called capsaicinoids. Perucka and Materska (2001) described these compounds as vanillylamides of branched fatty acids, with 9-11 carbons, of which capsaicin and dihydrocapsaicin are the predominant compounds that are responsible for the spiciness of pepper. These bioactive chemicals are used in the food industry, and production of defensive sprays. Moreover, capsaicin has shown great potential as chemo preventive agent against cancer diseases (Oyagbemi *et al*. 2010). Genetic variations in capsaicin contents or pepper hotness have been reported by several studies. Deng *et al*. (2009) and Sanathombi and Sharma (2008) reported that genotypes from the *Capsicum annuum* species have shown lesser amount of capsaicin than those reported for other capsicum species. In addition, peppers are known for their rich ascorbic acid content and this ascorbic acid is one of the powerful antioxidant agents that are having a number of health promoting functions (Bosland and Votava 2000; Davey *et al*. 2000; Libby and Aikawa 2002; Szeto *et al*. 2002; Vanderslice *et al*. 1990). So far different methods have been used for the quantification of these bioactive compounds. Scoville test method has been used for determining pungency and advanced instrumental technologies for the determination of capsaicin content. Likewise, the 2, 4-dinitrophenylhidrazine (DNPH) and High Performance Liquid Chromatography (HPLC) methods have been used for determination of ascorbic acid content in different fruits and vegetables.

Nowadays vegetables with high nutritional content and quality are of consumer's interest. Hence, understanding the variations of these bioactive compounds across peppers germplasms has great importance in developing better quality pepper varieties through crop improvement program. Therefore, the present study was undertaken to determine the capsaicin (pungency) and ascorbic acid content of seven chillies and paprika accession/ varieties using High Performance Liquid Chromatography (HPLC).

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MATERIALS AND METHODS

The experiment was carried out at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India. Seven planting materials (chillies and paprika) were used for the study. Standard horticultural practices recommended for chillies and paprika cultivations were uniformly applied to all genotypes.

Standards and Chemical Reagents: Pure Capsaicin and Ascorbic acid chemicals obtained from the micro analytical laboratory, Tamil Nadu Agricultural University, India, were used as reference standards. HPLC grade chemicals were used for the mobile phase and all the other reagents used in the analysis were analytical grade.

Capsaicin analysis: Capsaicin extractions were performed using the technique described by Estrada *et al*. (2001) with some modifications. First fully ripe fruits samples were allowed to dry at 60°C for 30 hours and crushed into powder before the extraction. Next, one gram of these pepper powders mixed with 5mL of HPLC grade acetonitrile was heated in a water bath at 80 °C for five hours and stirring was done at 30 minute intervals. Finally, the supernatants were rotated at 6000 rpm for 20 minutes and passed through 0.45 µm acrodiscs before they were injected into the HPLC column. The extracts were analysed using Agilent 1200 series HPLC equipped with auto sampler and Diode Array Detector (DAD) detector that was set at 280nm. Extracts of 20µl were injected into Zorbax eclipse C-18 (150mm X 4.6 I.D) column that was set at a temperature of 30ºC. An isocratic mobile phase was used for the separation of capsaicin at a flow rate of 1ml/minute. A mixture of acetonitrile, water, acetic acid at a ratio of 100: 100: 1 was used as mobile phase and pure solution of capsaicins were used as reference standard.

Scoville Heat Unit Conversions: Capsaicin contents were converted to Scoville Heat Units by multiplying the pepper capsaicin content (grams of capsaicin per grams of pepper dry weight) by the coefficient of the heat value for capsaicin (1.6×10^7).

Ascorbic acid analysis: Ascorbic acid of fully ripe fruit was extracted using the method described by Zhang and Hamauzu (2003). Two grams of fresh pepper fruits pulps sliced into small pieces were homogenized with 15 ml of 5% Meta phosphoric acid for 15 minutes. The mixture was filtered through Whatman No. 4 filter paper with two successive extractions of the remaining residues. The filtrates were pooled and centrifuged at 4,000g for 10 min. Finally the volume of supernatant was made up to 50 ml and filtered through 0.45µm. HPLC analysis was done according to the method reported by Hedau *et al*. (2008) with some modifications. The analysis was done on Agilent 1200 series HPLC equipped with Diode Array Detector (DAD) detector, auto sampler, and Zorbax Eclipse C-18 (150mm X 4.6 I.D) with the temperature set at 30^{O}C. Twenty micro litres of the extract from each sample was injected into the column using an automatic injector and an isocratic mobile phase at flow rate of 1.0 ml/min was used for the separation of ascorbic acid. Acetonitrile and Methanol mixture at a ratio of 50:50 were used as eluent and the standard solutions of ascorbic acid (0.1–1.0 mg/mL) were used to make calibration curves at 254nm.

RESULT AND DISCUSSION

In the study, the capsaicin and ascorbic acid contents of seven peppers genotypes were determined using high performance liquid chromatography. Based on the chromatograms generated from the standard solutions and pepper samples peaks for capsaicin (Fig 1) and ascorbic acid (Fig 3) were eluted at 5.34 minute and 3 min after injection, respectively.

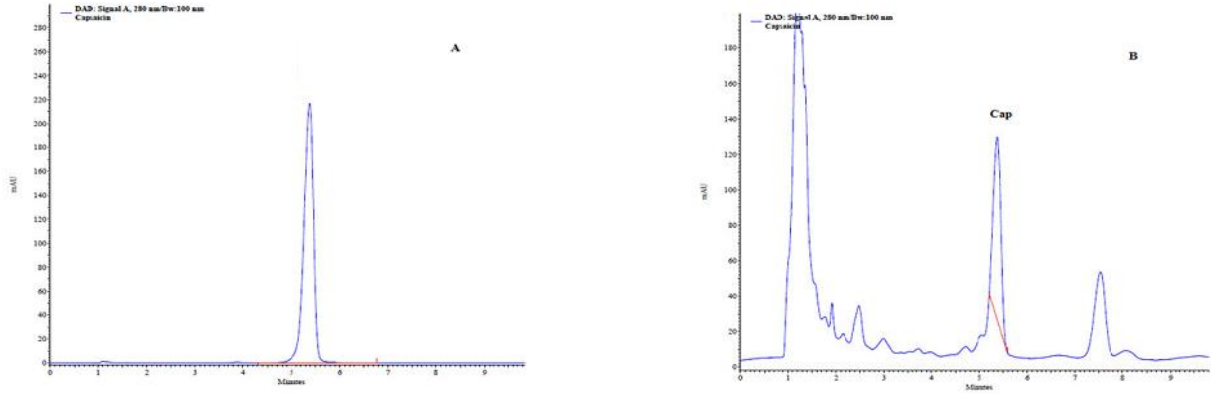

Figure 1. HPLC chromatograms of capsaicin standard (A) and CF1 accession (B). Cap = capsaicin peaks

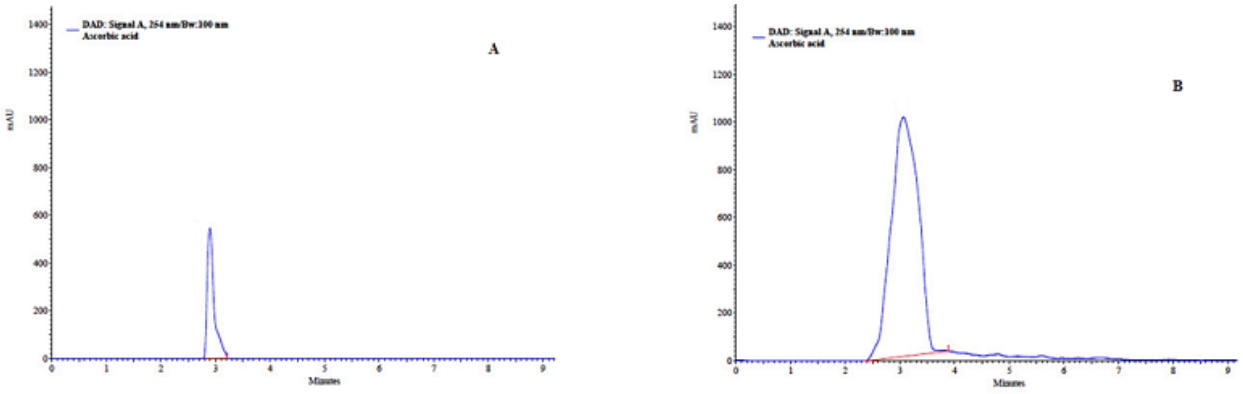

Figure 2. HPLC chromatograms of ascorbic acid standard (A) and Bayadagi Kaddi variety (B).

Capsaicin contents, Scoville Heat Units (SHUs) and ascorbic acid concentrations obtained from dried fruits samples are shown in Table 1. The accession CF1 showed the highest capsaicin content (445mg/100g DW) with equivalent pungency of 71,200 SHUs and the accession CA97 showed moderate capsaicin content (297mg/100g DW) and 47,600 SHUs pungency levels. On the contrary, the lowest capsaicin content (29mg/100g DW) and pungency levels (4,672 SHUs) were obtained from Arka Abhir variety. Similar to other reports the accession from *Capsicum frutescens* species showed the highest capsaicin content and was found to be hotter than all the *Capsicum annuum* accession/varieties described in this study (Antonious and Jarret 2006; Gnayfeed *et al*. 2001; Juliana *et al*. 1997; Mathur *et al*. 2000). In addition CF 1 showed higher capsaicin content than those reported for Tabasco variety (378.5mg/100g DW) which belongs to the *C. frutescens* species but it showed lower capsaicin content than the Orange Habanero variety (663.9mg/100g DW) from *C. chinense* species (Garceas-Claver *et al*. 2006). According to Weiss (2002) all the chilli accessions/varieties were classified as highly pungent. Though, KTPL19, Bayadagi Kaddi and Arka abhir were released as paprika peppers with high colour value (Prasath *et al*. 2007) they showed relatively higher capsaicin content and according to Korel *et al*. (2002) they should be grouped as chilli peppers. Hence, further breeding program should be taken up in order to lower the capsaicin content of these high colour paprika varieties. In the presented data ascorbic acid concentrations ranged from 55.3 to 189 mg/100g FW with an average vitamin C content of 113.2 mg/100 g FW (Table 1).

Table 1. Capsaicin content, pungency level and ascorbic acid content of four chilli (CA97, CCH CF1 K1) and three paprika (KTPL19, Arka Abhir, Bayadagi Kaddi) cultivars.

Pepper varieties/ accession	Capsaicin mean values	SHU	Ascorbic acid (mg/100g)
CA97	2.975 ± 0.177[b]	47,600	55.3 ± 7.3[c]
CCH	1.705 ± 0.135	27,280	172.9 ± 4.8
CF 1[a]	4.45 ± 0.21	71,200	61.8 ± 9.29
K1	2.275 ± 0.11	36,400	117.6 ± 2.6
KT PL19	1.155 ± 0.24	18,480	119.5 ± 4.24
Arka Abhir	0.292 ± 0.006	4,672	76.0 ± 7.07
Bayadagi Kaddi	0.608 ± 0.12	9,728	189.4 ± 10.32

[a] belongs to Capsicum frutescens species and the rest are from Capsicum annuum

[b] mg/g ± SD, [c]mg/100g ± SD, n=2

The Bayadagi kaddi variety showed the highest ascorbic acid content and the lowest ascorbic acid content was obtained from CA 97 accession. The ascorbic acid content obtained from the Bayadagi kaddi variety was higher than the vitamin C content found from Guindilla pepper variety (168.5 mg/100 g FW). However, this value is lower than those reported for Red Lamuyo (293 mg/100 g FW) and Red California (348 mg/100 g FW) varieties (Guil-Guerrero *et al*., 2006; Rodriguez-Burruezo *et al*. 2009). It is also necessary to underline

the high ascorbic acid content of CCH (172.9 mg/100gmFW), K1 (117.6 mg/100gmFW), and KTPL 19 (119.5 mg/100gmFW) accessions/varieties which can be used as potential sources for vitamin C. Therefore, an average adult person could meet the daily Recommended Nutrient Intakes (RNI) of 45 mg/day, FAO/WHO (2001) by consuming 40g of these pepper fruits.

CONCLUSION

The variability presented in the pepper germplasm for capsaicin and ascorbic acid content can be exploited for breeding of cultivars with improved nutritional qualities. All the chilli types can be used as a potential source of capsaicin, especially the CF1 accession. Likewise, Bayadagi Kaddi can be used as a source of vitamin C for enhancing the nutritive value of human diets.

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