*Available online at* www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5791

**ISSN: 2320 – 7051**

*Int. J. Pure App. Biosci.* **6 (1):** 1322-1327 (2018)

International Journal of Pure & Applied Bioscience

*Research Article*

OPEN ACCESS

# Quantification of Capsaicin and Ascorbic Acid Content in Twenty Four Indian Genotypes of Chilli (*Capsicum annuum* L.) by HPTLC and Volumetric Method

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Received: 25.09.2017 | Revised: 30.10.2017 | Accepted: 4.11.2017

**ABSTRACT**

*Capsaicin and ascorbic acid contents of twenty four Indian chilli varieties/accessions from Capsicum annuum (2011/CHIVAR-1,2011/CHIVAR-2, 2011/CHIVAR-3, 2011/CHIVAR-4, 2011/CHIVAR-5, 2011/CHIVAR-6, 2011/CHIVAR-7, 2011/CHIVAR-8, 2011/CHIVAR-9, KA-2(C), LCA-334(C), UTKAL AVA(L.C), 2012/CHIVAR-2, 2012/CHIVAR-3, 2012/CHIVAR-4, 2012/CHIVAR-5, 2012/CHIVAR-6, 2012/CHIVAR-8, 2012/CHIVAR-9, 2013/CHIVAR-1, 2013/CHIVAR-2, 2013/CHIVAR-3, 2013/CHIVAR-4, UTKAL RASHMI(L.C) were determined using High Performance Thin Layer Liquid Chromatography (HPTLC) and Volumetric method. Based on their pungency value, all the chilli accession/varieties were classified as highly pungent peppers. The accession 2011/CHIVAR-6 recorded maximum capsaicin content 50890mg/100g DW followed by 2011/CHIVAR-5 45800mg/100g DW, LCA-334 44540mg/100g DW and 2011/CHIVAR-7 40300mg/100g DW were observed to be similar and better than Utkal Ava 1070mg/100g DW KA-2 2890mg/100g DW, Utkal Rashmi 3430mg/100g DW. The minimum 500mg/100g DW was recorded by the genotype 2011/CHIVAR-8. In case of green chilli, maximum ascorbic acid was recorded in 2011/CHIVAR-2 (112 mg 100 $g^{-1}$) closely followed by 2012/CHIVAR-4 (101.33 mg 100 $g^{-1}$), 2011/CHIVAR-6 (77.33 mg 100 $g^{-1}$) and 2011/CHIVAR-9 (74.67 mg 100 $g^{-1}$). Lowest ascorbic acid content was found in 2013/CHIVAR-2 (26.67 mg 100 $g^{-1}$). Similarly, Ascorbic acid content of red ripe fruits of the chilli genotypes, had a wide range of variation (45.33 mg to 317.33 mg 100 $g^{-1}$ FW). Maximum ascorbic acid content of 317.33 mg 100 $g^{-1}$ FW was recorded in 2011/CHIVAR-3 followed by 2011/CHIVAR-4 (304 mg 100 $g^{-1}$ FW) and 2011/CHIVAR-1 (290.67 mg 100 $g^{-1}$) which were better than the rest. The genotype KA-2 recorded minimum ascorbic acid content of 45.33 mg 100$g^{-1}$ FW. The variability in capsaicin and ascorbic acid content presented in the pepper germplasm can be exploited for breeding cultivars with improved nutritional qualities.*

***Key words:*** *Capsicum annuum, Pungency, Vitamin C.*

**Cite this article:** Pradhan, K., Nandi, A., Das, A., Sahu, N., Senapati, N., Mishra, S.P., Patnaik, A. and Pandey, G., Quantification of Capsaicin and Ascorbic Acid Content in Twenty Four Indian Genotypes of Chilli (*Capsicum annuum* L.) by HPTLC and Volumetric Method, *Int. J. Pure App. Biosci.* **6(2):** 1322-1327 (2018). doi: http://dx.doi.org/10.18782/2320-7051.5791

## INTRODUCTION

Hot chilli peppers (genus Capsicum) are among the most heavily consumed spices throughout the world which are known for their pungency, pigment and nutrition content[3]. The pungency of peppers is attributed to the presence of a group of compounds called capsaicinoids. They are produced as secondary metabolites in chilli peppers, probably as deterrents against herbivores. Perucka and Materska[11], 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 for production of defensive sprays. Moreover, capsaicin has shown great potential as chemo preventive agent against cancer diseases[10]. Besides its use as a food additive in various spicy cuisines, Capsicum (due to its capsaicin content) is currently used for various other therapeutic purposes such as asthma, coughs, sore throats, to relieve toothaches, counter-irritant balm for external application, to alleviate pain, arthritis, diabetes etc.

Capsaicin is mostly located in the vesicles or vacuole like sub-cellular organelles of the epidermal cells of the placenta in the pod[9]. It is also not evenly distributed in pepper fruit. In general, the highest capsaicin concentrations are found in the ovary and in the lower flesh (tip) and the lowest capsaicin content can be found in the seeds[14]. Capsaicinoids are produced in glands on the placenta of the fruit[2]. The seeds are not the source of pungency but they occasionally absorb capsaicin because they are in close proximity to the placenta.

There is a rapidly growing economic significance in a wide array of food products, medicine, industry, law enforcement and pest control [3], owing to the popularity and familiarity of products containing Capsicum. There is scope for regulating capsaicin biosynthesis in Capsicum genotypes to meet the demands of food, pharma and cosmetics industries. The chilli fruit develops greater pungency in tropical countries like India, Africa and Tropical America than in the cold regions. Genetic variations in capsaicin contents or pepper hotness have been reported by several studies[12,5].

For quantitative estimation and quality control, fast and simple methods are needed that do not require very modern equipment. Chromatographic methods, in particular Thin Layer Liquid Chromatography/ High Performance Thin Layer Liquid Chromatography and High Performance Liquid Chromatography are used extensively for material product identification, quantification and purification[13]. Since it offers several advantages, TLC is widely used for rapid analysis of drugs and drug preparations out of the many chromatographic methods presently available[16,18].

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[1,4,7,15,17]. 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, Volumetric method 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 twenty four chillies accession/ varieties using High Performance Thin Layer Liquid Chromatography (HPTLC) and Volumetric method.

## MATERIAL AND METHODS

The experiment was conducted in randomized block design with three replications under All India Coordinated Research Project on

Vegetable Crops at Horticultural Research Station, Orissa University of Agriculture and Technology, Bhubaneswar (East and SE Coastal Plain Zone, $20^0$15'N latitude and $85^0$52' E longitude). The experimental material comprised of 24 chilli genotypes grown during winter season of 2013-14. Thirty five days old seedlings were transplanted in the main field with a spacing of 50cm x 30cm. Recommended cultural practices were uniformly followed to raise the crop successfully.

The twenty four diverse genotypes of chilli selected for the present work were 2011/CHIVAR-1, 2011/CHIVAR-2, 2011/CHIVAR-3, 2011/CHIVAR-4, 2011/CHIVAR-5, 2011/CHIVAR-6, 2011/CHIVAR-7, 2011/CHIVAR-8, 2011/CHIVAR-9, 2012/CHIVAR-2, 2012/CHIVAR-3, 2012/CHIVAR-4, 2012/CHIVAR-5, 2012/CHIVAR-6, 2012/CHIVAR-8, 2012/CHIVAR-9, 2013/CHIVAR-1, 2013/CHIVAR-2, 2013/CHIVAR-3, 2013/CHIVAR-4, KA-2(C), LCA-334(C), Utkal Ava(L.C.) and Utkal Rashmi (L.C.). The genotypes were supplied by the Indian Institute of Vegetable Research, Varanasi, India and Orissa University of Agriculture & Technology, Bhubaneswar, India. Each of the genotypes was grown till full maturity. The fruits were picked when they just turned red. They were dried under shade for two weeks. Fruits from 5 different plants of each genotype were sampled together in duplicate. They were kept in a preset oven at temperature of 45 ±2 ˚C for four days till they were brittle. The chillies were then powdered and sieved after separating their stalks. A fine powder of chillies was collected from the bottom sieve and stored in air tight containers. Then analysis of sample was performed through HPTLC after appropriate modifications in the methodology as reported by Wagner and Bladt[18].

**Preparation of Gibb's reagent**

Gibb's reagent, or 2,6-dichloroquinone-4-chloroimide (assay 99%), was procured from Loba Chemie Ltd., Mumbai. Reagent for derivatization was prepared by dissolving 500 mg of Gibb's reagent in 100 ml methanol.

Capsaicin standard, as well as the samples were spotted on pre-coated silica gel 60F254 plates (Manufacturer: E.Merck), using CAMAG Linomat V sample applicator. The mobile phase employed was Chloroform: Methanol: Acetic acid (9.5: 0.5: 0.1, v/v/v). The plates were developed up to 80 mm in CAMAG twin trough development chambers (20 x 10 cm), post chamber saturation of 15 minutes. The plate was allowed to dry in air, dipped in Gibb's reagent, air dried again and later exposed to ammonia vapours in a twin trough chamber. As a result, blue violet zones were visible due to the spontaneous reaction which remained for 2-5mins. Densitometric scanning of the plates was performed at 254nm , using CAMAG TLC Scanner 3. The values of the areas obtained for the standards were plotted as a function of the concentrations of the standard applied on each track. The regression coefficient, relative standard deviation, slope and intercept on the Y-axis were calculated by WINCATS software. Based on the value of the relative standard deviation, the Limit of Detection (LOD) and the Limit of Quantification (LOQ) were then calculated. The calibration graph so obtained was used to quantify the capsaicin content in each sample (Figure 1).

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

**Ascorbic acid analysis:** This analysis was performed with composite (composited over 3 replications) samples of 24 genotypes. Ascorbic acid content in fruit was estimated by volumetric method. 5 ml of standard ascorbic acid (100 µ g/ml) was taken in a conical flask containing 10 ml 4% oxalic acid and was titrated against 2,6-dichlorophenol indophenol dye. The appearance and persistence of pink colour was taken as end point. The amount of dye consumed ($V_1$ml) is equivalent to the amount of ascorbic acid. 5 ml of sample (prepared by taking 2.5g of fruit in 100 ml 4% oxalic acid) was taken in a conical flask having10 ml of 4% oxalic acid and titrated

against the dye ($V_2$ml). The amount of ascorbic acid was calculated using the formula,

Ascorbic acid (mg/100 g) = (0.5 mg/$V_1$ml)×($V_2$/5 ml)×(100 ml/Wt. of sample)×100

## RESULTS AND DISCUSSION

In the present study, capsaicin and ascorbic acid contents of twenty four genotypes of chilli were determined using high performance liquid chromatography (HPTLC) and Volumetric method respectively. The HPTLC plate developed for analysis of the extracts of all the twenty four genotypes of chilli are shown in Plate no. 1 & 2. Volumes were loaded and amount of fractions spotted. The graphical relationship was developed between peaks of the areas and different genotypes along with standard.

Capsaicin contents, Scoville Heat Units (SHUs) and ascorbic acid concentrations obtained are shown in Table 1. The accession 2011/CHIVAR-6 recorded maximum capsaicin content of 50890 mg/100g DW followed by 2011/CHIVAR-5(45800mg/100g DW), LCA-334 (44540mg/100g DW) and 2011/CHIVAR-7 (40300mg/100g DW) which were observed to be similar and better than Utkal Ava (1070 mg/100g DW) KA-2 (2890mg/100g DW) and Utkal Rashmi (3430mg/100g DW). The minimum of 500 mg/100g DW was recorded by the genotype 2011/CHIVAR-8.

In case of capsaicin analysis, a wide range of variation (500 to 5089 mg/100g DW) was observed among the 24 genotypes of chilli evaluated. Yatung *et al*. similarly reported that capsaicin content of chilli genotypes contributed maximum towards divergence. Weiss stated in his study that high level of capsaicin content was noticed in most of the genotypes of chilli as compared to a few others.

In case of green chilli, maximum ascorbic acid was recorded in 2011/CHIVAR-2 (112 mg 100 $g^{-1}$) closely followed by 2012/CHIVAR-4 (101.33 mg 100 $g^{-1}$), 2011/CHIVAR-6 (77.33 mg 100 $g^{-1}$) and 2011/CHIVAR-9(74.67mg100 $g^{-1}$). Lowest ascorbic acid content was found in 2013/CHIVAR-2 (26.67 mg 100 $g^{-1}$). It conforms to the range of ascorbic acid reported by Litoriya *et al.*[8] in green chilli. Ascorbic acid content of red ripe fruits of the chilli genotypes, had a wide range of variation (45.33 mg to 317.33 mg 100 $g^{-1}$ FW). Maximum ascorbic acid content of 317.33 mg 100 $g^{-1}$ FW was recorded in 2011/CHIVAR-3 followed by 2011/CHIVAR-4 (304 mg 100 $g^{-1}$ FW) and 2011/CHIVAR-1 (290.67 mg 100 $g^{-1}$) which were better than the rest. The genotype KA-2 recorded minimum ascorbic acid content of 45.33 mg 100$g^{-1}$ FW. In general, the ascorbic acid content of red ripe chilli was found to be higher than that of green chilli, which is in conformity with the findings of Khyadagi *et al.*[6].

**Table 1: Capsaicin content, pungency level and ascorbic acid content of twenty four chilli genotypes**

| Genotypes | Capsaicin values(mg/100g DW) | Scoville Heat Units (SHU) | Ascorbic acid(mg/100g FW) | |
|---|---|---|---|---|
| | | | Green | Red |
| Standard | 39110 | 6257600 | | |
| 2011/CHIVAR-1 | 1270 | 203200 | 56.00 | 290.67 |
| 2011/CHIVAR-2 | 32240 | 5158400 | 112.00 | 234.67 |
| 2011/CHIVAR-3 | 25055 | 4088000 | 56.00 | 317.33 |
| 2011/CHIVAR-4 | 37230 | 5956800 | 64.00 | 304.00 |
| 2011/CHIVAR-5 | 45800 | 7328000 | 53.33 | 157.33 |
| 2011/CHIVAR-6 | 50890 | 8142400 | 77.33 | 208.00 |
| 2011/CHIVAR-7 | 40300 | 6448000 | 58.67 | 168.00 |
| 2011/CHIVAR-8 | 500 | 80000 | 53.33 | 152.00 |
| 2011/CHIVAR-9 | 2960 | 473600 | 74.67 | 242.67 |
| KA-2(C) | 2890 | 462400 | 29.33 | 45.33 |
| LCA-334(C) | 44540 | 7126400 | 40.00 | 152.00 |
| UTKAL AVA(L.C) | 1070 | 171200 | 34.67 | 96.00 |
| 2012/CHIVAR-2 | 31290 | 5006400 | 58.67 | 130.67 |
| 2012/CHIVAR-3 | 21600 | 3456000 | 53.33 | 61.33 |
| 2012/CHIVAR-4 | 23510 | 3761600 | 101.33 | 128.00 |
| 2012/CHIVAR-5 | 19790 | 3166400 | 58.67 | 98.67 |
| 2012/CHIVAR-6 | 530 | 84800 | 48.00 | 50.67 |
| 2012/CHIVAR-8 | 830 | 132800 | 48.00 | 93.33 |
| 2012/CHIVAR-9 | 740 | 118400 | 37.33 | 53.33 |
| 2013/CHIVAR-1 | 4210 | 673600 | 34.67 | 48.00 |
| 2013/CHIVAR-2 | 7100 | 1136000 | 26.67 | 64.00 |
| 2013/CHIVAR-3 | 5580 | 892800 | 37.33 | 101.33 |
| 2013/CHIVAR-4 | 7890 | 1262400 | 34.67 | 64.00 |
| UTKAL RASHMI(L.C.) | 3430 | 548800 | 37.33 | 85.33 |

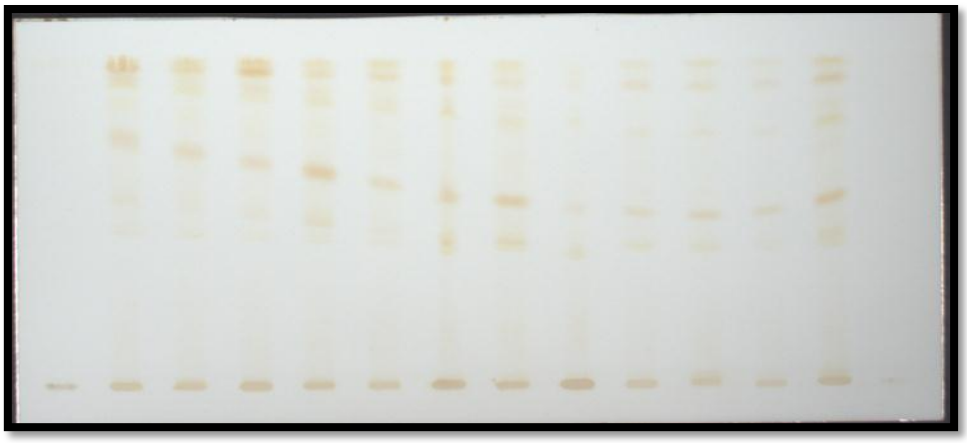

**Plate No.1[White light] Picture of bands in silica gel plate after dipping through Gibb's reagent under monochromatic light**

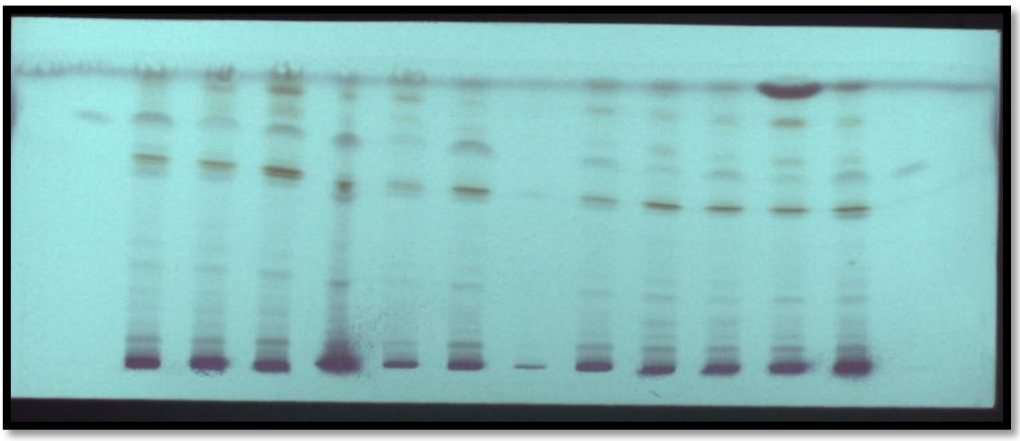

**Plate No. 2 [UV light] Picture of bands in silica gel plate after passing through Gibb's reagent, dry air and ammonia vapour successively under ultra-violet light**

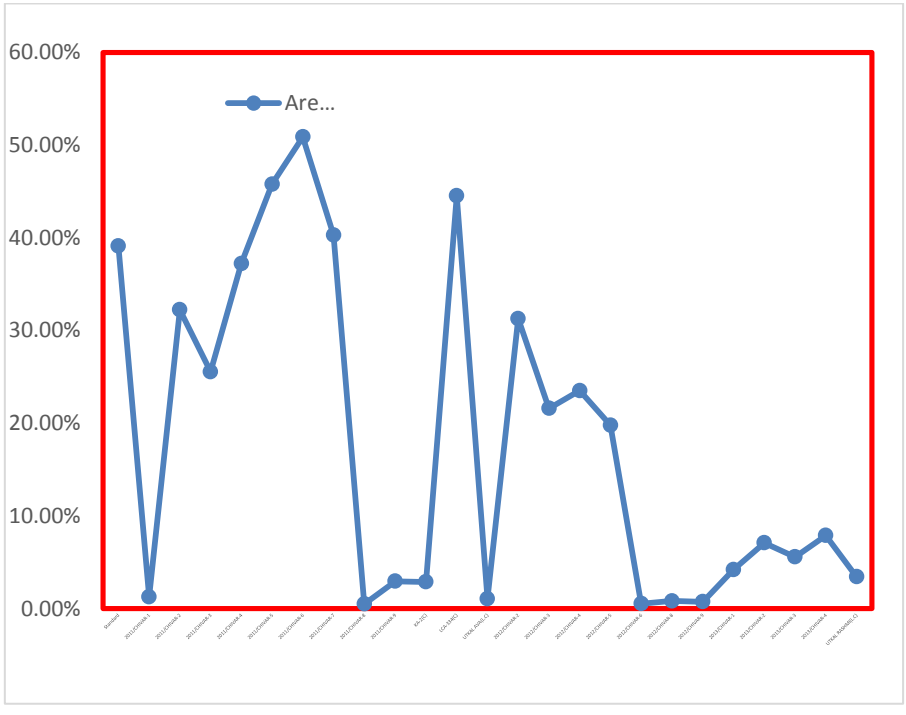


**Fig. 1: Comparison of area of genotypes with reference to standard capsaicin**

## CONCLUSION

The variability presented in the pepper germplasm for capsaicin and ascorbic acid content can be exploited for breeding of new varieties with improved nutritional qualities. All the chilli genotypes can be used as a potential source of capsaicin, especially the 2011/CHIVAR-6. Likewise, 2011/CHIVAR-2 and 2011/CHIVAR-3 can be used as a source of vitamin C for enhancing the nutritive value of human diets for green and red chilli respectively.

**Acknowledgements**

To Indian Institute of Vegetable Research (I.C.A.R.), Varanasi and Orissa University of Agriculture & Technology, Bhubaneswar, for financial support and provision of physical facilities.

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