ORIGINAL ARTICLE

Variation of capsaicinoids in chilli (*Capsicum frutescens* L.) cultivars with the maturity of fruits in middle hill conditions of western Himalayas

Hemant Kumar Pandey, Deendayal, Vandna Pandey, Tribhuwan Pant, Zakwan Ahmed
Defence Institute of Bio-Energy Research (DRDO), Panda, Pithoragarh, Uttarakhand, India

The three chilli (Capsicum *frutescens* L.) cultivars viz. chilli DARL 210, Chilli Long Black and Sartoria were grown in middle hill climatic conditions of western Himalayas at an altitude of 5,500 feet above the sea level. The variation in total capsaicinoids (Capsaicin and dihydrocapsaicin) was studied from budding to full maturity stage of fruits. The fruits were harvested at every fifteen days interval from flower budding (stage zero) to full maturity stage (stage seven). The total capsaicinoids were evaluated with the help of HPLC. It can be concluded from the study that, to achieve maximum pungency (total capsaicinoids) in these cultivars, the chilli cv. DARL 210 should be harvested at the stage of full maturity (after 105 days). Whereas, Chilli long black should be harvested after 75 days and cultivar Sartoria in between 60 to 90 days from flower budding stage. As far as pungency among these cultivars is concerned DARL 210 was the most pungent followed by Chilli Long black, while Sartoria was found least pungent cultivar.

Key words: Chilli (*Capsicum frutescens* L.), Capsaicin, Dihydro-capsaicin, High performance liquid chromatography, Scolville heat unit

INTRODUCTION

Chilli or hot pepper is a commonly cultivated Solanaceous crop of India. Different cultivars of chilli are being grown for vegetables, spices, pickles, condiments,etc. It is mainly used for its pungency and colouring properties. India is the largest producer and consumer of chilli among the other major producer of the world. India contributes about 25% to the total world production and remained at the first position in terms of international trade by exporting 20% from its total production. India exports different forms of hot peppers like chill powder, dried chilli, pickled chillies and chilli oleoresins. The production during 2008–2009 is nearly 13 lakh tonnes. The hotness or pungency of chilli is due to presence of a group of compounds called capsaicinoids.[1] Among capsaicinoids, capsaicin and dihydrocapsaicin are present in major quantities, while homocapsaicin, nordihydrocapsaicin and homo-dihydrocapsaicin are present in very small quantities.[2] The capsaicin is produced by the glands at the junction of placenta and the pod wall, distributed unevenly throughout the pod and found maximum in placental tissues.[3] It is a powerful and stable alkaloid which remains unaffected by cold or heat and remains unchanged despite of time, cooking or freezing. It has no colour, flavour or odour. Capsaicin stimulates the mucous membrane of mouth and stomach and increase the secretion of gastric juices.[4] The sensation of heat and pain in the mouth is the result of stimulation of local heat receptors in the skin and mucous membrane by capsaicin. It is a trigeminal stimulant, which is important in gustatory physiology. Now a days capsaicin is also used to relieve pain, the prolong application of capsaicin causes desensitisation of sensory nerve, responsible for the pain. Keeping the commercial and pharmaceutical importance of capsaicinoids in view, a study was conducted at the Defence Institute of Bio-energy Research (DRDO), Pithoragarh to analyse total capsaicinoids in three chilli cultivars viz. DARL 210, Chilli Long Black and Sartoria during different stages of fruit development, so that the stage having maximum capsaicinoids can be detected, in the middle hill climatic conditions of western Himalayan region. The cultivars viz. DARL-210, Chilli Long Black and Sartoria are famous for their pungency (due to capsaicinoids) and red colour because of the pigment capsanthin. Cultivars DARL-210 is the selection from local cultivars. It is perennial in habit and falls under scotch Bonnet group. Chilli Long Black and Sartoria are the introduction from Defence Research Laboratory, Tezpur, Assam, these are also perennial in habit and cime under Chiltepin peppers. The percentage of capsaicinoids in dried red capsicum powder is an important index for quality of the products, which is widely used as species by the world food Industry for pungency.

The accumulation of capsaicinoids is especially associated with development stages of fruit, like other alkaloids, it accumulates and later undergoes a rapid turnover and degradation during fruit development.[5]

Address for correspondence: Dr. Hemant Kumar Pandey, Scientist-D, Defence Institute of Bio-energy Research (DRDO), Pithoragarh – 262 501, Uttarakhand, India. E-mail: hkpandey5@rediffmail.com
Received: 22-06-2009; **Accepted:** 04-12-2009; DOI: 10.4103/0973-8258.69177

MATERIALS AND METHODS

Collection and Cultivation of Capsicum Varieties

Three varieties of Capsicum *frutescens* viz. DARL 210, Chilli long black and Sartoria were collected from Horticultural Division of Defence Institute of Bio-Energy Research, Pithoragarh and nursery of these varieties was raised. The seedlings were transplanted with a row to row and plant-to-plant spacing of 75 cm×75 cm and recommended agronomical practices were strictly followed.

Sampling Procedure and Preparation of Samples

The flowers of each cultivar were tagged. The pepper fruits were harvested at every fifteen days interval from the budding stage (Stage 0) to 105 days (Stage 7). Average height and weight of fruits at different stages were recorded. The harvested fruits were dried at 50°C in oven and grounded into fine powder and kept in airtight containers at room temperature, prior to extraction. For extraction of capsaicinoids, 1 gram finely grounded powdered samples of all three cultivars were dissolved in 10 ml of acetonitrile with two hours shaking on a shaker. After that, flask was left overnight at room temperature. The supernatant was filtered with Whatman filter paper No. 1, thereafter by 0.45 μm filter paper and with a 10 ml disposable syringe into 1 ml glass vial. The volume of injection in HPLC was taken 10 μl.

Estimation of Capsaicinoids by HPLC

The samples were analysed using Water's Quaternary Gradient HPLC system equipped with Waters 717 auto sampler, temperature controller, Waters - 996 photodiode array detector and Millennium^{32} software for data processing. Reverse phase HPLC was carried out on a Spherisorb RP C-18 ODS- 1 column (150 mm × 46 mm), having particle size 5 μm. A pre- column guard cartridge Spherisorb RP C-18 was also used. The capsaicinoids were determined under uniform HPLC conditions: column temp. 30°C, flow rate 1.5 ml/min. and run time was 15 minutes. The mobile phase was isocratic with solvent combination (Acetonitrile: Water containing 1.00 % acetic acid in 70:30). The capsaicin and dihydro-capsaicin were purchased from Sigma chemicals Co. (St Louis Mo) and used for retention time verification and instrument calibration. The mean retention time of capsaicin and dihydro-capsaicin under above conditions were 2.20 and 2.55 minutes, respectively. Data were statically analysed by Panse and Sukhatme method.[6]

RESULTS AND DISCUSSION

The physical parameters of fruits i.e., fruit length (cm) and fruit weight (g) were summarised in Table 1. It is evident from table that the gradual increase in fruits length was observed up to 60 days thereafter, it remained stable. In chilli cultivar Sartoria, maximum fruit length (6.90 cm) was found, followed by (6.80 cm) in Chilli long black and minimum (3.00 cm) in cv. DARL 210. There was an increasing trend in fruit weight in all three-chilli cultivars from stage 1 to stage 7. Maximum fruit wt. (3.70 g) was observed in chilli DARL 210 followed by Chilli long black (2.80 g) while, minimum wt. (2.70 g) was recorded in cultivar Sartoria. The fruit maturation process is accompanied by loss of water, it was reported that the dry matter increases with maturity of fruit.[7] The variation of capsaicinoids contents with the development of fruits were also studied by some earlier workers.[8-10] The results pertaining to variation in total capsaicinoids, capsaicin and dihydrocapsaicin with the maturity of fruits, in three chilli cultivars are cited in Table 2. The HPLC chromatograms of these cultivars at the stage of their maximum pungency are cited in Figure 1. The data of capsaicinoids content are cited in percent as well as in Scoville Heat Units (SHU).

Wilbur Scoville developed as scale in 1912 to measure the heat levels of the chilli peppers. In the original, Scoville test, a panel of volunteers would be asked to determine what dilution of chilli pepper solution no longer cause burning discomfort in the mouth. Approximately one part per million of "heat" is equivalent to 1.5 Scoville units. The total capsaicinoids have been calculated on percent dry weight basis. For conversion of present capsaicinoids to SHU results been multiplied with 1,50,000 (i.e., 1.5 lakh).

It was revealed from data that chilli cv. DARL 210 showed gradual increase in total capsaicinoids in every 15 days i.e., absent in first 15 days (stage 1) to 1.843% (276500 SHU) in

Table 1: Variation in quantitative characters with the maturity of fruits

Cultivar name	Stage	Av. fruit length (Cm)	Av. fruit weight (g)
DARL-210	First	1.10	0.30
	Second	1.80	2.10
	Third	2.30	2.50
	Fourth	2.80	3.00
	Fifth	2.90	3.30
	Sixth	3.00	3.60
	Seventh	3.00	3.70
Chilli long black	First	2.70	0.50
	Second	4.60	1.50
	Third	5.70	1.80
	Fourth	6.50	2.00
	Fifth	6.80	2.20
	Sixth	6.80	2.60
	Seventh	6.80	2.80
Sartoria	First	2.80	0.50
	Second	5.70	1.50
	Third	6.60	1.70
	Fourth	6.80	1.90
	Fifth	6.90	2.00
	Sixth	6.90	2.50
	Seventh	6.90	2.70

105 days (stage 7). During this period capsaicin content varied from 0.48% in 30 days to 0.930% in 105 days old fruit, while dihydro-capsaicin varied from 0.416% in 30 days to 0.913% in 105 days.

In cv. Chilli long black total capsaicinoids ranged from 0.002% in 15 days to 1.219% (182950 SHU) in 75 days, after that downward trend was observed. In 90 days total capsaicinoids were reduced to 0.931% and almost same concentration (0.945%) was reported till 105 days. In this cultivar capsaicin varied from 0.002% in first 15 days to maximum 0.945% after 105 days. While, dihydro-capsaicin ranged from 0.293% in 30 days old fruit to maximum 0.645% in 75 days old fruit, thereafter, the concentration decreased in the fruits and at 105 days whole dihydro-capsaicin was converted into capsaicin.

In cv. Sartoria total capsaicinoids varied from 0.068% in first 15 days to 0.772% (115800 SHU) in 75 days, then its concentration decreased to 0.582% after 105 days .Minimum capsaicin content 0.032% was recorded in 15 days and maximum 0.392% in 75 days old fruits, then it was declined to 0.343% and 0.260% in 90 days and 105 days old fruits, respectively. Dihydro-capsaicin on the other hand varied from 0.036 % in 15 days to 0.391 % in 90 days after that its concentration decreased slightly up to 105 days.

The decrease of capsaicinoids contents in Chilli Long Black and Sartoria at later stage might be due to senescence stage of fruits, which started after 75 days in cultivar Chilli Long Black and Sartoria because in capsicum fruit, peroxidase enzyme activity increases at the time when the concentration of capsaicinoids started to decrease because this enzyme involved in the degradation of capsaicinoids.

Gross *et al.*,[8] studied bio-chemical changes associated with the ripening of hot pepper (*Capsicum annuum* L.cv. Chooraehong) fruit and studied ethylene production, changes in galactose and arabinose level and β-galactosidase enzyme activity from immature green to the red ripped stage of fruit. Estrada *et al*,[9] studied changes in capsaicin, lignin, free phenolic and peroxidase pattern in pepper fruits *Capsicum annuum* cv. Padron in a week interval (from 14 days to 42 days after flowering). The level of capsaicinoids remained low for 21 days but 28 days after flowering capsaicinoids increased moderately and finally at the end of development cultivar exhibited a dramatic increase in capsaicinoids level with the highest values found in the fifth stage i.e., 42 days after flowering.

Contreras- Padilla *et al*,[10] experimented evolution of capsaicinoids in the three varieties of hot Chilli peppers viz Habanero (*Capsicum chinense* Jacq.), Dearbol (*C. annuum*

Table 2: Variation in capsaicinoids content with the maturity of fruits (Dry weight basis)

Name of chilli cultivar	Stages	Capsaicin (%)	Dihydro-capsaicin (%)	Total capsaicinoids (%)	Scoville heat units
DARL-210	First	NA	NA	NA	NA
	Second	0.480	0.416	0.896	1,34,500
	Third	0.652	0.512	1.164	1,74,650
	Fourth	0.757	0.739	1.497	2,24,500
	Fifth	0.850	0.709	1.559	2,33,850
	Sixth	0.846	0.763	1.609	2,41,400
	Seventh	0.930	0.913	1.843	2,76,500**
Cd at 1%		0.0099	0.011	0.017	
Cd at 5%		0.0071	0.008	0.012	
CV		0.6180**	0.8070**	0.5800**	
Chilli long black	First	0.002	NA	0.002	320
	Second	0.317	0.293	0.610	91,500
	Third	0.321	0.391	0712	1,06,800
	Fourth	0.450	0.420	0.870	1,30,500
	Fifth	0.574	0.645	1.219	1,82,950
	Sixth	0.504	0.427	0.931	1,39,700
	Seventh	0.945	NA	0.945	1,41,750**
Cd at 1%		0.0071	0.012	0.008	
Cd at 5%		0.0051	0.008	0.006	
CV		0.6480**	1.0300**	0.4490**	
Sartoria	First	0.032	0.036	0.068	10,300
	Second	0.204	0.180	0.385	57,800
	Third	0.192	0.215	0.407	61,100
	Fourth	0.365	0.376	0.742	1,11,300
	Fifth	0.392	0.380	0.772	1,15,800
	Sixth	0.343	0.391	0.735	1,10,200
	Seventh	0.260	0.322	0.582	87,300
Cd at 1%		0.0052	0.0082	0.0068	
Cd at 5%		0.0037	0.0059	0.0048	
CV		0.6840**	1.5200**	0.5220**	

** - Significant

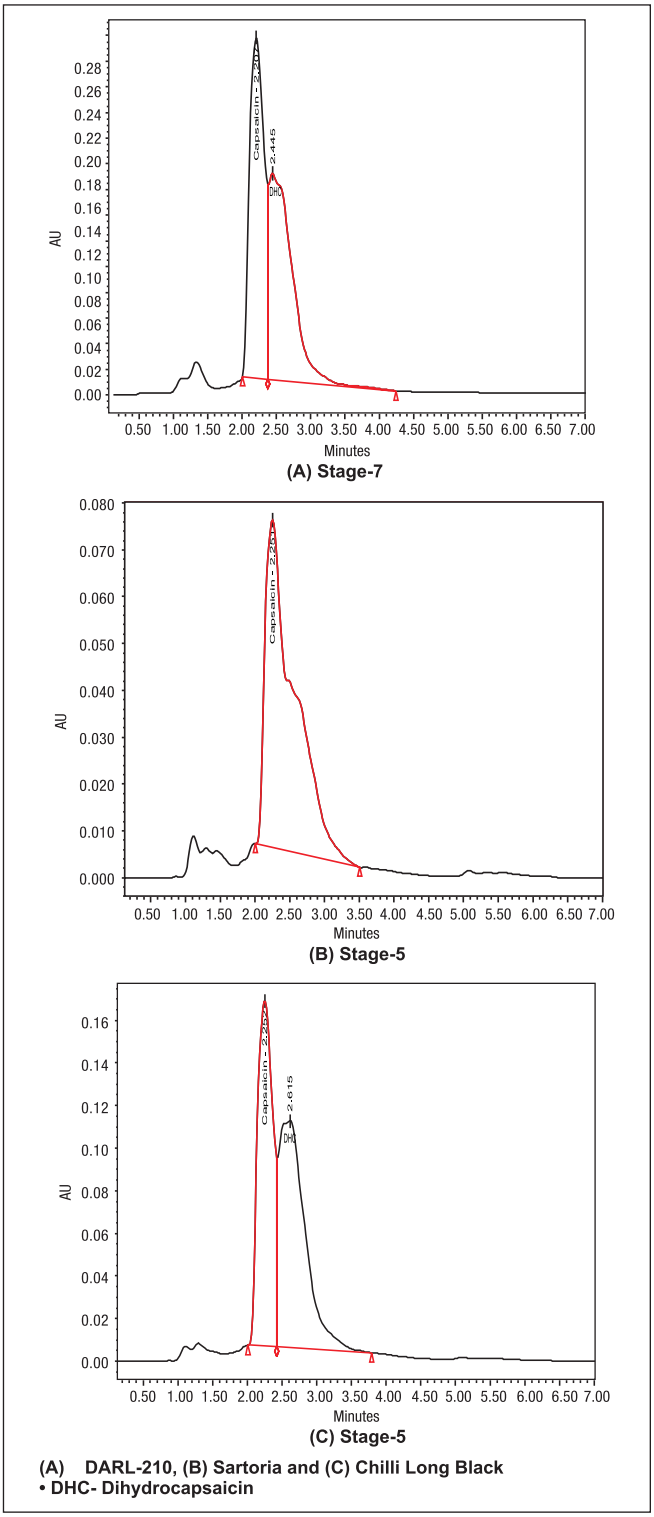

Figure 1: Capsaicinoids content in capsicum cultivars

var. Annuum) and Piquin (*C. annuum* var. Aviculare) during the development, maturation and senescence of fruits. Capsaicinoids were more abundant in the fruits of Habanero followed by Dearbol and the by Piquin. Capsaicin was higher than dihydrocapsaicin in all three varieties. Capsaicinoids, capsaicin and dihydrocapsaicin increased continuously and reached a peak after 45–50 days from fruit set in Habanero and Dearbol and after 40 days in Piquin and than declined. He observed peroxidase activity increased at the time when the concentration of capsaicinoids started to decrease. There was an inverse relationship between the evolution of capsaicinoids and peroxide activity that might indicate that this enzyme is involved in capsaicinoids degradation.

In present study, Chilli cultivers DARL-210, Chilli Long Black and sartorial took 105 days for attaining maturity from the flowering stage. Gradual increase in total capsaicinoids contents were observed in Chilli cultivar DARl-210 from 15 days to 105 days. While, in Chilli Long Black and Sartoria increase was observed up to 75 days then decrease in capsaicinoids was observed. These results are of similar trend as reported by earlier workers.

CONCLUSION

Hence, it can be concluded from the study that to achieve maximum capsaicinoids content for medicinal as well as industrial use from these cultivars, the chilli cv. DARL 210 should be harvested at the stage of full maturity (after 105 days). Whereas, Chilli long black should be harvested after 75 days and cultivar Sartoria in between 60 to 90 days from flowers budding stage of the plants. Among chilli cultivars DARL - 210 was found most pungent having highest total capsaicinoids contents followed by cv. Chilli Long black whereas, cv. Sartoria was found least pungent. For pharmaceutical/commercial point of view cv. DARL 210 was found best. By large scale cultivation of this cultivar farmer can get good return of their crop.

REFERENCES

1. Estrada B, Pomar F, Diaz J, Merino, Bernal FA. Evaluation of capsaicinoids in *Capsicum annuum* L. Var. annuum cv. Padron at different growth stages after flowering. Capsicum Newslett 1997;16:60-4.
2. Tewari A, Kausik MP, Pandey KS, Dangi RS. Adaptability and production of hottest chilli variety under Gwalior agro-climatic conditions. Curr Sci 2005;88:1545-6.
3. Rowland BJ, Villalon B, Burns EE. Capsaicin production in Sweet Bell and pungent Jalapeno peppers. J Agric Food Chem 1984;31:484-7.
4. Andrews J. Peppers. Austin: University of Taxas Press; 1984.
5. Iwai K, Suzuki T, Fujiwake H. Formation and accumulation of pungent principle of hot pepper fruits, capsicum and its analogues, in *Capsicum annuum* var. *annuum* cv. Karayat subusa at different growth stages after flowering. Agric Biol Chem 1979;43:2493-8.
6. Panse VG. Statistical methods for agricultural workers. New Delhi: Indian Council of Agricultural Research; 1961.
7. Govindaraju VS. *Capsicum* – Production, technology, chemistry and quality: PartI History, botany, cultivation and primary processing. Crit Rev food Sci Nut 1985;22:109-16.
8. Gross KC, Watada AE, Kang MS, Kim SD, Kim KS, Lee SW. Biochemical changes associated with the ripening of hot pepper

fruits. Physiol Plant 1986;66:31-36.
9. Estrada B, Maria AB, Diaz J, Pomar F, Merino F. Fruit Development in *Capsicum annuum*: Changes in Capsaicin, lignin, free phenolics and Peroxidase pattern. J Agric Food Chem 2000;48:6234-9.
10. Contreras-Padilla M, Yahia EM. Changes in capsaicinoids during development, maturation and senescence of chilli peppers and relation with peroxidase activity. J Agric Food Chem 1998;46:2075-9.

Source of Support: Nil, **Conflict of Interest:** None declared.