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Cultivar variation for capsaicinoid content in some processed products of chilli

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ABSTRACT

Determination of capsaicinoids content in various products from seven chilli cultivars was made. Capsaicin, the major element among capsaicinoids, is found primarily in the fruit of *Capsicum* to which it provides spicy flavor. Extraction of capsaicin in the present study was done using acetonitrile as the solvent, and High Performance Liquid Chromatography was used for its quantification. Whole dried-fruits of 'Bhut Red' (*Capsicum chinense*) showed the highest concentration of capsaicin (2.59%) and level of pungency (4,40,000 SHU), whereas, salted mash of 'Lemon Drop' (*Capsicum baccatum)* had the lowest capsaicin concentration (0.07%) and pungency level (12,000 SHU). As capsaicinoids are important in food and pharmaceutical industries, developing products from selected cultivars of chilli with high pungency and high capsaicinoid content will prove useful in order to ensuring health security.

Key words: Capsaicinoids, capsaicin, High Performance Liquid Chromatography, HPLC, SHU

INTRODUCTION

The immense horticultural, agricultural and biological diversity has made chilli globally important as a fresh and processed vegetable, and as a source of ingredients for sauces and powders besides its use as a food colorant (Boseland Vatava 2000). Lately, a demand for value-added products prepared from chilli like dried pods, flakes, powder, color oleoresin, pungent oleoresin, etc. has been steadily increasing. There is a wide range of chilli products, based on whole or ground chilli, entering world trade. Most of these products are traded on the basis of their level of pungency. One attribute, typical of chillies, is its pungency, resulting from a direct effect of its capsaicinoid compounds on pain receptors in the mouth and throat (Gibbs and Yahia, 2006). Quantification of these pungent compounds is an important index of pepper quality (Contreras-Padilla *et al*, 1998). The primary capsaicinoid in chilli pepper is capsaicin, followed by dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin. Capsaicin and dihydrocapsaicin, the two most potent capsaicinoids, account for approximately 90% of the capsaicinoids in chilli pepper fruit (Bernal *et al*, 1993). Considerable variation in capsaicin content has been reported by Cherian (2000); with the amount increasing in the order of: green fruit < ripe fruit < sundried fruit (Ahmed *et al,* 1987). However, this content decreases with the degree of drying, as well as during storage (Gbolade *et al*, 1997). The content may vary from 0.34 to 0.78% whole fruit on dry-weight basis (Gibbs *et al*, 2006). Govindarajan and Ananthakrishna (1974) found 0.12% capsaicin in 'Mysore' variety of chilli, and 0.7% in 'Guntur' variety. In another experiment, 32 accessions of hot chillies were evaluated for capsaicin content, all of which had a high capsaicin content ranging from 1.20 to 3.74% (Manju and Shreelathakumary, 2002).

The first reported, reliable measurement of chilli pungency was Scoville Organoleptic Test (Scoville, 1912). This test used a taste panel of five individuals who evaluated a chilli sample and recorded its heat level. The samples were then diluted until pungency could be no longer detected orally. This dilution is measured in terms of Scoville Heat Unit (SHU). There are five levels of pungency classified as: non-pungent (0-700 SHU), mildly pungent (700-3,000 SHU), moderately pungent (3,000-25,000 SHU), highly pungent (25,000-70,000 SHU), and very highly pungent (Ã 80,000 SHU) (Weiss, 2002). However, with time, the Scoville Organoleptic Test (SOT) has been replaced with various instrumental methods. Among these HPLC provides an accurate and efficient analysis of content and type of capsaicinoids present in a sample (Collins *et al*, 1995).

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MATERIAL AND METHODS

Collection of plant material

Seeds of 'Bhut Red', 'Bhut Chocolate', 'Mem Jolokia', and 'Khorika Jolokia' were collected from the fields of local farmers in the north eastern part of India. Seeds of 'Shillong Cherry' were collected from the Shillong market. Those of 'Lemon Drop' and 'Goronong' were received from Pepper King, Lise-Meitner-Str. 5, 38268 Broistedt, Germany. Seedlings of the stated chilli types were raised in a nursery during *rabi* season (2007-2008) and, subsequently, planted in the open condition at Experimental Farm, Department of Horticulture, Assam Agricultural University, Jorhat, India. Each variety was raised in a $6m^2$ plot, at a spacing of 75cm x 60cm. The crop received timely management practices as per recommended package of practices devised by Assam Agricultural University. Fresh, mature fruits from five plants, in each cultivar were selected randomly and placed in polythene bags for transport under refrigerated conditions to Quality Control and Post-Harvest Technology Laboratory, Assam Agricultural University, for further analysis.

Product preparation

Five products, namely, whole dried-pods, flakes, powder, natural paste and salted mash, were prepared from different cultivars of chilli.

(a) Whole dried-pods: Fresh, ripe pods were collected from the field and dried in a unither cabinet dryer at 60°C for about 8 hours. Dried pods were then packed into polypropylene bags and then kept under dark at ambient condition.

(b) Flakes: Dried, ripe pods were first destalked, and then crushed with seeds in a Willey mill. Size of the flakes prepared was about 8-10mm.

(c) Powder: Dried, ripe pods were destalked and powdered in an electrical grinder. Using an 80 mesh sieve, the ground powder was sieved, collected and packed in polypropylene pouches and placed under ambient conditions.

(d) Natural paste: Ripe chilli fruits were collected from the field, washed well and air-dried. Pods were destalked and ground to a fine paste in an electrical grinder. The paste thus prepared was filled into retortable pouches and processed in an autoclave under 15lb pressure at 121°C for 30 minutes.

(e) Salted mash: Ripe fruits were at first destalked, washed well and made into a paste. Salt @ 15% (non-iodized) was added to the paste and mixed. The product was then sterilized in glass bottles under anaerobic conditions and allowed to mature.

Moisture content

Moisture content in each product was determined by oven-drying at 120p C as per AOAC (1984). The samples were weighed in an aluminum pan, and weight of the dry sample was recorded. Dry weight was calculated as:

$$\text{Moisture content (dry basis)} = \frac{\text{Initial weight-Final weight}}{\text{Final weight}} \times 100\%$$

Scoville Heat Test

One gram of the material was weighed and mixed in 50ml of ethyl alcohol. The mixture was allowed to stand for 24 hours, with occasional shaking. Serial dilution of the clear supernatant was made with 5% solution of sugar in distilled water. Then, 5ml of the diluted solution was swallowed by individual judges, and presence or absence of a distinct pungency in the throat or mouth was noted (Scoville, 1912). Degree of dilution indicated pungency of the pepper, and rating was done in Scoville units. Heat level is based on this dilution, rated in multiples of 100 SHU.

Sample extraction

About 25g of whole dried-pods and flakes in each cultivar were ground together with seeds into a powder. Then, 2g of the powder was mixed in 25ml of ethanol in a 50ml conical flask. The mixture was refluxed in a round bottom flask for 3 hrs, with a water condenser, vertically. The solution was cooled, filtered and diluted to 100ml in a volumetric flask with ethanol, and used for further analysis.

As for salted mash and paste, 2g of the sample was extracted in ethanol, using Sohxlet extraction, for 30min. at 60p C. The supernatant was then filtered through 0.45mm PTFE membrane and the volume made up to 100ml with ethanol. A 10µl aliquot was used for each HPLC injection. The chilli extract was compared to standard capsaicin solutions (Krishnamurthy *et al*, 1999)

Estimation of capsaicinoids by High Performance Liquid Chromatography (HPLC)

Capsaicin content was determined using HPLC (High Performance Liquid Chromatography) as per AOAC Official Method 995.03 (1995) with a UV detector. 8-Methyl-N-Vanillyl-6-nonenamide standard (65% purity) was taken as standard capsaicin (Sigma Chemical Company). Separation of capsaicinoids was accomplished on Waters

HPLC–Empower system equipped with Waters 600 pump, C_{18} column of size 300x4.5mm packed with 5µm particles, Waters 2489 Dual absorbance detector, detection made at 280nm. An isocratic mobile phase, consisting of 1% acetic acid in water and acetonitrile in 60:40 ratio (v/v), was used. The elution was allowed at a flow-rate of 1.5ml/min with injection volume of 20µl of the sample solution, at ambient temperature.

Scoville Heat Unit Conversion

Capsaicin content was converted into Scoville Heat Units by multiplying dry-weight capsaicin content per gram of pepper, by the coefficient of heat value for capsaicin (which, from literature, is 16) (Todd *et al,* 1977).

Chemicals used

All standard solutions were prepared in analytical grade Type I water (Milli-Q Synthesis, Millipore, Bedford, MA, USA). Capsaicin (8-methyl-*N*-vanillyl-*trans*-6-nonenamide) (97%), and, acetonitrile and acetic acid were of HPLC grade purchased from Qualigen Fine Chemicals, Mumbai, Spectrochem Pvt. Ltd., Mumbai, and Himedia Laboratories Pvt. Ltd., Mumbai, respectively.

Statistical analysis

Completely Randomized Design (CRD) was planned for this experiment, with three replications. Mean difference at 5% significance was carried out by Duncan's Multiple Range Test (DMRT). Graph were prepared in Microsoft Excel (MS Office version 2003)

RESULTS AND DISCUSSION

The aim of our work was to assess capsaicinoid content and pungency level of various value-added products of seven economically important chilli cultivars. Retention of desirable qualities in the processed product makes the product acceptable in the market. However, along with these, an optimum level of moisture is necessary for safe storage. Lee and Howard (1999) reported that moisture content in dried chilli ranged from 10% to 14%. Product evaluation in Table 1 reveals variable moisture content, where, very high moisture content was recorded in the paste and salted mash, compared to that in dried fruits, flakes or powder. Moisture content in the products ranged from 8% to 10% in dried fruits, 4%-6% in powder, 6%-8% in flakes, 58%-67% in paste and 46%-64% in the salted mash. These moisture variations were a critical factor in determining product quality in terms of pungency level.

Most chilli products are valued on the basis of level of their pungency, resulting from a direct effect of capsaicinoid compounds. Quantification of these compounds is an important index of quality. In our study peaks were identified by comparing retention time of each component to standard components. Diverse pungency levels were found among the products. As per results in Tables 2 and 3, Scoville Heat Units (SHU) and the corresponding capsaicinoid content in different cultivars were significantly affected by the product from chilli cultivars. SHU is a traditional organoleptic method for pepper evaluation, as, it provides a better indicator of the level of pungency, but is considered less precise (Collin *et al,* 1995). Our results indicated significant variation among different products in their pungency level. All the cultivars, excepting Lemon Drop, were classified as 'very highly pungent', with their pungency level Ã 80,000 SHU (Table 2). Among the products developed, whole dried-fruits and the powder of 'Bhut Red' recorded highest pungency level (4,40,000 SHU), which was *at par* with that of 'Bhut Chocolate' while, SHU was much lower in the paste and salted mash. Salted mash of 'Lemon Drop' contained least amounts of SHU (12,000 SHU).

Table 1. Moisture content (%) in various processed products of chilli cultivars (dry-weight basis)

Cultivar	Moisture content $(\%)_{db*}$				
	Whole dried-fruit	Powder	Flakes	Paste	Salted mash
Bhut Red	9.0	4.5	7.2	64.6	55.5
Bhut Chocolate	9.3	5.1	8.3	63.3	54.8
Mem	9.5	6.8	6.5	59.5	49.3
Khorika	8.3	6.1	6.0	58.7	46.8
Shillong Cherry	10.3	5.4	8.1	60.3	51.2
Goronong	8.9	6.3	8.3	66.7	61.1
Lemon Drop	9.3	6.9	8.8	65.0	63.7
S.Em. (±)	0.9	0.3	0.4	1.5	0.9
CD *(P=0.05)*	1.9	0.8	0.8	3.2	1.9

***** $_{db}$= dry-weight basis

Table 2. Scoville Heat Unit values in some processed products of chilli cultivars

Cultivar	Scoville Heat Unit (SHU)				
	Whole dried-fruit	Powder	Flakes	Paste	Salted mash
Bhut Red	4,40,000	4,40,000	4,10,000	3,15,000	3,00,000
Bhut Chocolate	4,30,000	4,35,000	4,00,000	3,10,000	3,00,000
Mem	1,40,000	1,40,000	1,30,000	1,00,000	90,000
Khorika	95,000	95,000	85,000	70,000	60,000
Shillong Cherry	90,000	90,000	75,000	60,000	55,000
Goronong	65,000	65,500	60,000	35,000	32,500
Lemon Drop	25,000	25,000	20,000	15,000	12,000
S.Em. (±)	4764.31	4764.31	2432.14	2458.61	2059.6
CD *(P=0.05)*	9528.61	9528.61	5864.27	5325.48	4231.2

Table 3. Total capsaicinoid content in processed products of some chilli cultivars

Cultivar	N		C		D		Total	
	SHU	mg g^{-1}	SHU	mg g^{-1}	SHU	mg g^{-1}	SHU	mg g^{-1}
Whole dried-fruit								
Bhut Red	11310.4	706.9	414504.0	25906.5	141372.8	8835.8	565489.6	35343.1
Bhut Chocolate	14068.8	879.3	410816.0	25676.0	139550.4	8721.9	562515.2	35157.2
Mem	6040.0	377.5	144952.0	9059.5	50329.6	3145.6	201321.6	12582.6
Khorika	5217.6	326.1	97299.2	6081.2	33912.0	2119.5	136428.8	8526.8
Shillong Cherry	2094.4	130.9	86019.2	5376.2	36153.6	2259.6	124665.6	7791.6
Lemon Drop	849.6	53.1	24428.8	1526.8	9913.6	619.6	35403.2	2212.7
Goronong	2246.4	140.4	52412.8	3275.8	19467.2	1216.7	74875.2	4679.7
							S.Em(±)	1854.4
							CD *(P=0.05)*	3977.4
Powder								
Bhut Red	10760.0	672.5	392758.4	24547.4	134507.2	8406.7	538025.6	33626.6
Bhut Chocolate	133972.8	873.3	385846.4	24115.4	137811.2	8613.2	537054.4	33565.9
Mem	6219.2	388.7	149249.6	9328.1	51822.4	3238.9	207291.2	12955.7
Khorika	5289.6	330.6	92563.2	5785.2	34380.8	2148.8	132233.6	8264.6
Shillong Cherry	2574.4	160.9	86838.4	5427.4	39337.6	2458.6	128752.0	8047.0
Lemon Drop	705.6	44.1	24342.4	1521.4	10230.4	639.4	35278.4	2204.9
Goronong	2227.2	139.2	54920.0	3432.5	17068.8	1066.8	74216.2	4638.5
							S.Em(±)	1709.1
							CD *(P=0.05)*	3665.6
Flakes								
Bhut Red	12254.4	765.9	375785.6	23486.6	127644.8	7977.8	510577.6	31911.1
Bhut Chocolate	12755.2	797.2	371150.4	23196.9	126324.8	7895.3	510230.4	31889.4
Mem	5825.6	364.1	139798.4	8737.4	48540.8	3033.8	194164.8	12135.3
Khorika	4912.0	307.0	85942.4	5371.4	31921.2	1995.1	122774.4	7673.4
Shillong Cherry	2400.0	150.0	82747.2	5171.7	34777.6	2173.6	119923.2	7495.2
Lemon Drop	614.4	38.4	21169.6	1323.1	8897.6	556.1	30681.6	1917.6
Goronong	2131.2	133.2	53976.0	3373.5	16344.0	1021.5	71057.6	4441.1
							S.Em(±)	1182.8
							CD *(P=0.05)*	2536.9
Paste								
Bhut Red	7254.4	453.4	271859.2	16991.2	83371.2	5210.7	362484.8	22655.3
Bhut Chocolate	7249.6	453.1	268412.8	16775.8	81612.8	5100.8	357274.2	22329.7
Mem	2651.2	165.7	59211.2	3700.7	26512.0	1657.0	88376.0	5523.5
Khorika	1014.4	63.4	32972.8	2060.8	16740.8	1046.3	50728.0	3170.5
Shillong Cherry	913.2	57.7	29676.8	1854.8	15065.6	941.6	45656.0	2853.5
Lemon Drop	905.6	56.6	ND	ND	11972.8	748.3	12944.0	809.0
Goronong	1208.0	75.5	29796.8	1862.3	9260.8	578.8	40267.2	2516.7
							S.Em(±)	2541.3
							CD *(P=0.05)*	5298.6
Salted mash								
Bhut Red	7484.8	467.8	280691.2	17543.2	86078.4	5379.9	374256.0	23391.0
Bhut Chocolate	9358.4	584.9	280729.6	17545.6	84219.2	5263.7	374307.2	23394.2
Mem	3788.8	236.8	66310.4	4144.4	24628.8	1539.3	94728.0	5920.5
Khorika	2097.6	131.1	35137.6	2196.1	15209.6	950.6	52444.8	3277.8
Shillong Cherry	987.2	61.7	32060.8	2003.8	162776.8	1017.3	49315.2	3082.2
Lemon Drop	372.8	23.3	10940.4	683.8	4219.2	263.7	15628.8	976.8
Goronong	1267.2	79.2	31238.4	1952.4	9708.8	606.8	42214.4	2638.4
							S.Em(±)	1175.4
							CD *(P=0.05)*	2521.0

N – Nordihydrocapsaicin, C – Capsaicin, D – Dihydrocapsaicin, SHU-Scoville Heat Unit, ND-Not detected

Results obtained from organoleptic test were further confirmed by HPLC analysis. Generally, apart from genetic differences, quantity of capsaicinoids may vary with the processing method (Zewdie and Bosland, 2001). HPLC chromatogram of the capsaicin standard is shown in Fig. 1. Retention time for the constituents is 8 min for nordihydrocapsaicin, 8.8 min for capsaicin, and 13.4 min for dihydrocapsaicin. It is seen from Table 3 that whole

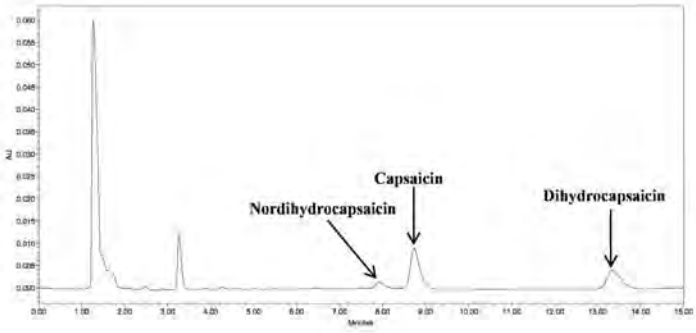

Fig. 1. HPLC chromatogram showing separation of the standards of various capsaicinoids (0.01mg/ml)

Table 4. Capsaicin content in some processed products (%) in chilli cultivars

Cultivar	Capsaicin content (%)				
	Whole dried-fruit	Powder	Flakes	Paste	Salted mash
Bhut Red	2.59	2.45	2.35	1.70	1.75
Bhut Chocolate	2.57	2.41	2.32	1.68	1.75
Mem	0.91	0.93	0.87	0.37	0.41
Khorika	0.61	0.58	0.54	0.21	0.22
Shillong Cherry	0.54	0.54	0.52	0.19	0.20
Lemon Drop	0.15	0.15	0.13	ND	0.07
Goronong	0.33	0.34	0.34	0.19	0.20

*% Capsaicin = SHU of capsaicin x 100
16x 10^6

dried-fruits of 'Bhut Red' contained the maximum total capsaicinoid content (5,65,489.6 SHU), closely followed by 'Bhut Chocolate' (5,62,525.2 SHU). Other cultivars like 'Mem', 'Kharika' and 'Shillong Cherry' contained slightly lesser amounts of capsaicinoid viz., 2,01,321.6, 1,36,428.8, and 1,24,665.6 SHU, respectively. Like whole dried-fruits, powder of 'Bhut Red' also contained the highest levels of capsaicinoids (5,38,025.6 SHU) than in the other cultivars. On the other hand, the paste of 'Lemon Drop' had the least capsaicinoid content (12,944 SHU), while, the maximal content was recorded in 'Bhut Red' (22,655.3 SHU). In salted mash, 'Bhut Chocolate' was found to be highly pungent, with 3,74,307.2 SHU. Among the individual components quantified, the highest amount of nordihydrocapsaicin was recorded in whole dried-fruits of 'Bhut Chocolate' (14,068.8 SHU), while capsaicin (4,14,504 SHU) and dihydrocapsaicin (1,41,372.8 SHU) were highest in dried fruits of 'Bhut Red'. Mincing fresh chilli pods diminishes their capsaicin, dihydrocapsaicin and nordihydrocapsaicin content, as reported by Orak and Demirci (2005). This could be of relevance in our results where least amounts of nordihydrocapsaicin (372.8 SHU), dihydrocapsaicin (4219.2 SHU) and capsaicin (10,940.4 SHU) were recorded in salted mash of 'Lemon Drop'.

Capsaicin percentage for different products varied with the cultivar. From Table 4, it can be observed that capsaicin content in the products ranged from 0.15% to 2.59% in dried fruits, 0.15%-2.45% in the powder, 0.13%-2.35% in the flakes, 0.19%-1.70% in the paste and 0.07%-1.75% in the salted mash, respectively. Whole dried-fruits of 'Bhut Red' were the most pungent, with a capsaicin content of 2.59%, whereas, even traces of this compound could not be detected in the paste of 'Lemon Drop'. A small amount of capsaicin (0.07%) was found in the salted mash of 'Lemon Drop'. Similar variation in capsaicin content (1.20%-3.74%) in different peppers has been previously reported by Manju and Shreelathakumary (2002).

The present investigation concludes that products of chilli cultivars retain their level of pungency irrespective of moisture content. More specifically, 'Bhut Red' and 'Bhut Chocolate' (*Capsicum chinense*) obtained from Assam were the most pungent among the chilli cultivars studied. All the products prepared from these two cultivars may be classified as 'very highly pungent' as their Scoville Heat Unit (SHU) values exceeded 80,000. This implies that, with the exception of 'Lemon Drop', all the products of chilli cultivars, especially 'Bhut Red' and 'Bhut Chocolate' can serve as potential sources of capsaicin in both the domestic and international markets.

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