Indian Journal of Natural Products and Resources
Vol. 8(1), March 2017, pp. 69-77

Effect of extraction methods and long term storage on capsaicinoids content of *Bhut Jolokia* fruits

Khomenendra Kumar Sarwa[1,2], Bhaskar Mazumder[1] and Mithun Rudrapal[1*]

[1]Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786 004, Assam, India
[2]Department of Pharmacy, Govt. Girls Polytechnic, Raipur 492001, Chhattisgarh, India

Received 28 December 2015; Revised 18 January 2017

The present investigation aimed to study effect of different methods of extraction and long term storage on capsaicinoids content of dried fruits of *Bhut Jolokia.* The powdered fruits of *Bhut Jolokia* were extracted by percolation, Soxhlet, microwave, and ultrasonication extraction methods using acetone as solvent. The extraction efficiencies of these methods were evaluated in terms of percentage yield of capsaicinoids content. Results indicate that Soxhlet extraction is more efficient in yielding capsaicinoids content than the percolation method. The extraction efficiency of microwave and ultrasonication methods was observed to be more as compared to Soxhlation, with equivalent yields of capsaicinoids content in a short time and at low temperature. Further, it is reported that capsaicinoids content is appreciably increased after six months of storage in dark atmosphere. Results suggest that ultrasonication followed by microwave extraction can be adopted in basic laboratory set up or small industrial scale for the extraction of *Bhut Jolokia* fruits, which would yield higher content of capsaicinoids than that of Soxhlet and percolation methods at a reasonably low processing cost. The study on enhancement of capsaicinoids content of dried fruits during long term storage would be very much useful to continue this practice on a commercial scale to meet the increasing demand of *Bhut Jolokia* in the global market.

Keywords: *Bhut Jolokia*, Capsaicinoids, Chili pepper, Extraction, Hybrid capsicum species, Storage.

IPC code; Int. cl. (2015.01)− A61K 36/00, A61K 36/81

Introduction

Bhut Jolokia, an indigenous variety of capsicum is a hybrid species of *Capsicum chinense* Jacq. and *C. frutescens* L. that is cultivated widely in North-eastern states of India namely Assam, Nagaland, Manipur and Arunachal Pradesh[1]. *Bhut Jolokia* (*Bhut* meaning *Ghost*, *Jolokia* meaning *Chili*) refers to the chili peeper that is the fruit of plants belonging to the genus Capsicum (Family Solanaceae)[1,2]. It was inducted in the Guinness Book of World Records in 2006 as the hottest/spiciest chili pepper in the world[3]. Because of possessing extreme hotness, this capsicum variety is popularly called by its vernacular name '*Bhut Jolokia*' in Assam (India). It is believed that a small amount of raw pepper (ripe capsicum fruits) or even sauce is so spicy that makes it barely edible by a normal person[4,5]. The matured fruits of *Bhut Jolokia* measure about 60-85 mm long and 25-30 mm wide with an attractive red color and a rougher, dented skin[1] (Plate 1). In North East India, it has been used as a dietary spice by different ethnic communities since time immemorial. Besides many commercial uses like pickle preparation, flavouring curries, and spice blends for food recipes, this chili pepper is also used in various traditional remedies for human ailments such as gastritis, arthritis, muscle pain, and chronic indigestion[4,6,7]. Moreover, semi-purified extracts of the *Bhut Jolokia* fruits are used in food and canning industries, increasing its demand in the global market[8].

Capsaicinoids represents a group of characteristic pungent compounds and are found invariably in fruits of capsicum (chili pepper). Hot and burning sensation of chili pepper is due to the presence of capsaicinoids in capsicum fruits[9,10]. Chemically, capsaicinoids are acid amides of vanillylamine (3-hydroxy-4-methoxy-benzylamide, vanilloid) with a C_9-C_{12} branched chain fatty acid. Five most naturally occurring capsaicinoids include capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homohydrocapsaicin[11,12]. Capsaicin and dihydrocapsaicin (Fig. 1) together constitute more than 80 % of total capsaicinoids content of capsicum[13]. These two major capsaicinoids are responsible for the total pungency of chili fruits[14].

*Correspondent author
E-mail: rudrapal.m03@gmail.com/rs_rudrapal@yahoo.co.in
Phone: 0373-2370254 (O)
Mobile: +91-9642785523

Plate 1 — *Bhut Jolokia* fruit

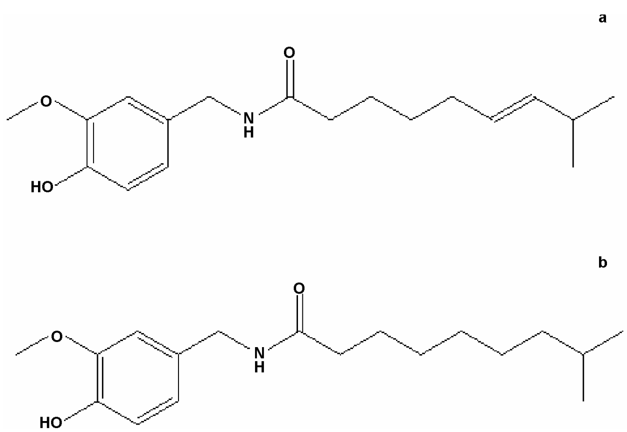

Fig. 1 — Structure of capsaicin (a) and dihydrocapsaicin (b)

Apart from capsaicinoids, flavonoids, phenolic acids, carotenoids, Vitamin A, and ascorbic acid are the other important constituents of capsicum. Capsaicinoids have been reported to possess certain pharmacological activities such as antiarthritic, analgesic, anti-inflammatory, antimicrobial, antiulcer, anticancer, and cardiovascular benefits[15-19]. It is attributed that the antioxidant property of capsaicinoids is primarily responsible for various pharmacological activities of capsicum fruits[20].

Literature report reveal that *Bhut Jolokia* fruits contain higher amount of capsaicinoids (2-3 %) as compared to other capsicum species (<1 %)[21]. Several research strategies have already been undertaken with the objective to improve the yield of capsaicinoids content in *Bhut Jolokia* fruits in order to meet its increasing commercial demand in both domestic and international market. They include agricultural manipulations with cultivation, harvesting, and storage of capsicum fruits[22-24] for better productivity, tissue culture methods for crop improvement[24], and biosynthetic modification[24] and chemical or enzymatic synthesis for increasing the yield[27-30]. Because of these, *Bhut Jolokia* fruits could be ideal for the extraction of pungent principles to get higher yield of capsaicinoids than any other chili fruits.

A number of solid-liquid extraction methods such as maceration[9], Soxhlet extraction[31], extraction by supercritical fluids[32,33], and enzymatic extraction[34, 35] have been used for the extraction of capsaicinoids from various capsicum fruits. They all require tedious sampling and cleaning up procedures. On a large scale basis, these extraction methods must be faster, cost-effective, and also reproducible. Moreover, since capsaicinoids are highly irritating substances, special precautionary measures are required to be taken while handling such substances to avoid any accidental hazards to health or environment either by direct contact or by inhalation, which may cause severe allergic reaction, skin toxicity or even death in severe toxic inhalation. In recent days, microwave[36,37] and ultrasound[38] like novel techniques are being used in the extraction of crude herbal drugs/plant materials.

It is believed that the potency of crude drugs or natural foods may get enhanced when stored properly for a longer duration. This is probably due to the fact that the concentration of bioactive principles in crude drugs/foods may increase during storage period. This phenomenon is usually seen with grain foods like rice, stored potatoes, dried herbs and spices, dry fruits, seasoning blends, and many more. For example, one year old stored rice has more market value than the newly arrived crop because of above reasons. Similar attributes can be made with homemade mixed pickles that have huge commercial demand in the domestic market. Local village people suggest that the hotness of *Bhut Jolokia* fruit increases when stored in tightly closed polythene bags under dark for about six months. Iwai and coworkers[11] reported that photooxidation of capsaicinoids may be prevented if capsicum pods are stored in dark atmosphere, while illumination induces it and thereby decreasing the capsaicinoids content. This may be due to the fact that illumination significantly restores the peroxidase enzyme involved in the oxidative degradation mechanism of capsaicinoids, which causes subsequent loss of pungent principle over storage period[39].

Several studies on other capsicum species having commercial importance have been carried out, which investigated the evaluation of different processing parameters such as time of harvesting, post harvesting treatment, and their effect on the yield of capsaicinoids content[14,22,32]. In an earlier study conducted by the authors, capsaicinoids content in different indigenous varieties of capsicum available in North-eastern region of India was investigated[9]. No work on evaluation of extraction methodologies, particularly, percolation, Soxhlation, microwave irradiation, and ultrasonication to assess their extraction efficiency in the yield of capsaicinoids content of *Bhut Jolokia* fruits has been conducted so far. The effect of long term storage conditions (atmospheric/stress) on capsaicinoids content of *Bhut Jolokia* has also not yet been reported.

In the present study, percolation, Soxhlation, microwave irradiation, and ultrasonication assisted extraction methods were evaluated to assess their efficiency in terms of yield of capsaicinoids content (mg/g of pure capsaicin) of *Bhut Jolokia* fruits. In addition, the effect of long term storage on capsaicinoids content of *Bhut Jolokia* fruits was also studied with the aim to assess the influence of several atmospheric factors on stability or degradation of capsaicinoids over storage period.

Materials and Methods

Plant materials

The matured ripe fruits of *Bhut Jolokia* were collected, dried under sunlight for about 7 days and stored (100 g) in well closed air-tight polythene bags in a dark room for about one month until further study. For long term storage, dried pods were stored under similar experimental conditions as mentioned above for a period of one year. The plant species of capsicum was authenticated by Botanical Survey of India, Shillong (BSI/ERC/2012/Plant identification/208).

Chemicals

Pure capsaicin (capsaicin 95 %, USP) was obtained as a gift sample from Chillies Export House, Virudhanagar, India. It contains capsaicin (59.87 %), dihydrocapsaicin (34.75 %) and nordihydrocapsaicin (3.21 %). Acetone was procured from Merck, Germany. Sodium hydroxide and phosphomolybdic acid were procured form Himedia, India.

Extraction of capsaicinoids[9,40]

Percolation

Dried *Bhut Jolokia* pods were coarsely powdered. The powder (10 g) was extracted using 100 mL of acetone for three successive cycles (30 h) at 40 °C. The extraction was performed in a thermostatic percolator with one channel peristaltic pump at a flow rate of 0.5 mL/min. The capsaicinoids content of the acetone extract was determined after completion of each cycle individually by spectrophotometric method.

Soxhlation

Coarsely powdered pods (10 g) were extracted in a Soxhlet apparatus using acetone as solvent (100 mL) until all the powdered material was completely exhausted (6 h). A constant temperature of about 65 °C was maintained throughout the extraction process. The extract so obtained was subjected to estimation of capsaicinoids content by spectrophotometric method.

Microwave extraction

Microwave extraction was carried out in a domestic microwave oven (Samsung MW73AD-B/XTL, 0.8 cu.ft./20 L, 230V- AC/50Hz, 800W) at variable time length and temperature. The powder (1 g) was extracted using 10 mL of acetone at 40, 50, and 60 °C from 10 to 60 min. The capsaicinoids content of the extract obtained under each experimental set up was estimated by spectrophotometric method.

Ultrasonication

In ultrasonication method, 1 g of the powder was extracted using 10 mL of acetone in an ultrasonicator (PCI 81 instrument, 33 +/- 3 KHz, 230 V- AC/50 Hz, 120W). The extraction was carried out for 60 min, with 2 min interval for every 10 min of operation. After 10, 20, 30, 40, 50, and 60 min of sonication temperatures were recorded as 35, 39, 41, 44, 46, and 47°C, respectively. The capsaicinoids content of the extract was estimated spectrophotometrically after completion of each sonication cycle.

Identification of capsaicinoids by TLC[41]

Thin layer chromatography (TLC) of the acetone extract was performed on a pre-coated silica gel GF-254 (10 μm layer thickness) using benzene-acetic acid (9:0.5) as a mobile phase. A spot was visualized in iodine chamber followed by UV detection at 254 nm. The detection of the spot intensified with the formation of red coloration by spraying 5 % sodium carbonate on the spot followed by gentle warming of the plate. Pure capsaicin was used as a reference standard. R_f values of the test extract were compared with that of the standard capsaicin.

Estimation of capsaicinoids content[42-44]

The capsaicinoids content of the acetone extract obtained after each extraction method was determined by spectrophotometric method as per the method reported by Sadasivam *et al*.[44] with minor modification. Acetone extract (2 mL) was transferred into a dry test tube and evaporated to dryness in a hot water bath. The residue was dissolved in a 5 mL of 0.5 % w/v aqueous solution of sodium hydroxide and 3 mL of 3 % w/v phosphomolybdic acid was added into it with gentle shaking for about an hour. The reaction mixture was centrifuged (Remi India) at 5000 rpm for 10 min in order to remove insoluble debris. The absorbance of the clear blue colored solution was then determined at 650 nm in a UV-visible spectrophotometer (Hitachi U-2001). The content of capsaicinoids in the extract was obtained from the calibration graph of the pure capsaicin. The capsaicinoids content is expressed as mg/g of pure capsaicin.

Effect of storage

Sun dried pods (100 g) were stored in polythene bags in a dark room for a period of one year. Stored pods were periodically measured for the weight loss. The percentage weight loss (in g of dry weight of capsicum fruits) was also recorded once in a month for one year. To evaluate the effect of storage on capsaicinoids content, stored pods was extracted by ultrasonication (drug to solvent ratio and experimental conditions were maintained same as described previously), and the capsaicinoids content of the extract was determined by spectrophotometric method.

Statistical analysis

Experiments were performed in triplicate and the results are expressed as mean±SD. Student's t test was performed.

Results and Discussion

Evaluation of the extraction methods

The extraction of capsaicinoids from dried *Bhut Jolokia* fruits was carried out by percolation, Soxhlation, microwave, and ultrasonication methods. The extraction efficiency of each extraction method was evaluated in terms of yield of capsaicinoids content considering different experimental conditions such as time and temperature. Acetone was used as a solvent of extraction since it is readily available, fairly non-toxic, volatile, and cheaper solvent. Moreover, capsaicinoids are freely soluble in acetone[11]. Prior optimization studies were carried out using different drug to solvent ratios. The drug to solvent ratio of 1:10 gave best extraction yield. Chloroform, ethyl acetate, methanol, and ethanol were also tested, but inadequate results in extraction yields were obtained with each of these solvent except acetone. Ethanol showed better results than other solvents and the yield was comparable to that of acetone. Ethanol was not used because of several issues like high viscosity, low diffusivity, and excise duty reasons. Ethanol extracted semi-purified dried extract has been reported to be waxy in nature as compared to the dried acetone extract[9].

The presence of capsaicin in the acetone extract was identified by test R_f value, i.e., 0.52 with that of the standard capsaicin[45].

In the percolation method, the capsaicinoids content was estimated to be 1.230±0.200 % after a 30 h long extraction process (after the completion of 3rd cycle) at 40 °C. On the other hand, the capsaicinoids content was found to be 2.453±0.022 % after 6 h of Soxhlet extraction at a temperature of 65 °C. The capsaicinoids content is expressed as mg/g of pure capsaicin. The value obtained in Soxhlet extraction was used as a reference for comparing the extraction efficiency of the remaining three methods. This is because Soxhlation is a widely useful scientific method for the extraction of plant material. Moreover, Soxhlation yielded best result amongst all. Results of percolation and Soxhlet extractions are presented in Table 1. The capsaicinoids content in percolation method is comparatively less than that of Soxhlet extraction.

In the microwave extraction, good yields of capsaicinoids were obtained after 40, 50, and 60 min of extraction at 40, 50, and 60 °C, respectively (Table 2). The yields of capsaicinoids content reached

Table 1— Capsaicinoids content in percolation and Soxhlet extraction

Percolation		Soxhlet etraction		
Cycle	Capsaicinoids content (%)*	Expt. No.	Capsaicinoids content (%)	Average content (%)*
1st	0.953±0.153	1	2.431	
2nd	1.127±0.210	2	2.453	2.453 ± 0.022
3rd	1.230±0.200	3	2.474	

Percolation and Soxhlation were completed in 30 h and 6 h, respectively

*Mean±SD (mg/g of capsaicin) of three experiments (n=3)

Table 2 – Capsaicinoids content in microwave extraction and ultrasonication

Time (min)	Microwave extraction Capsaicinoids content (%)*			Ultrasonication	
	40 °C	50 °C	60 °C	Capsaicinoids content (%)*	Temperature recorded (°C)
10	0.503±0.135	0.600±0.163	0.726±0.130	0.723±0.120	35
20	0.723±0.150	1.193±0.140	1.770±0.140	1.986±0.070	39
30	0.987±0.200	1.874±0.120	2.114±0.100	2.397±0.140	41
40	1.303±0.190	2.151±0.180	2.324±0.210	2.451±0.120**	44
50	1.905±0.120	2.305±0.110	2.426±0.220	2.451±0.190	46
60	2.353±0.200	2.452±0.110**	2.450±0.160	2.451±0.130	47

*Mean±SD (mg/g of capsaicin) of 3 experiments (n=3); **statistically significant p <0.001, compared to Soxhlet extraction

maximum after 40 min of extraction. After 60 min of extraction, values were constant till the end of the extraction process. The best extraction yield obtained was 2.452±0.022 % (p <0.001) after 60 min of extraction at 50 °C. In case of ultrasonication, the extraction for 40 min at 44 °C gave the best yield value with 2.451±0.022 % (p <0.001) of capsaicinoids content. Below 41 °C, the yields of capsaicinoids were poor, but there was no increase in yield at elevated temperatures such as 46 and 47 °C after 50 and 60 min of extraction, irrespectively. The extraction was less likely influenced by temperature since ultrasonication is independent of temperature. The temperature of the system increases with increase in sonication.

Results clearly reveal that the extraction efficiency of the traditional percolation and Soxhlation methods differ significantly from each other considering several parameters, mainly time of extraction and temperature. Soxhlation was found to be more efficient than the percolation process. Since Soxhlation is a continous hot percolation method, in this method of extraction the rate of mass transfer (between pods and solvent) is fairly facilitated by the rate of heat transfer[46] and henceforth, reduces the time and solvent for the total extraction process. On the contrary, the rate of mass transfer is comparatively slow in the percolation process, which in turn renders extraction to proceed slower and hence, less efficiently. Due to this reason, Soxhlation is highly efficient to extract maximum amount of capsaicinoids in a cycle, while, in percolation three successive cycles are insufficient for the extraction to yield capsaicinoids in good yield.

Modern methods like microwave and ultrasonication facilitate efficient extraction due to the fast removal of cuticular superficial waxes from plant material[47]. In microwave extraction, it is assumed that uniform heat distribution takes place throughout the plant material in a closed vessel, which results in extraction of higher amount of capsaicinoids at a temperature lower than the boiling point of acetone. On the other hand, ultrasound assisted extraction provides a rapid delivery of energy to the solvent that is forced directly on to the sample and thus causes disruptions of plant cells to run the extraction process very efficiently[48]. Ultrasound accelerate the process of swelling and hydration of plant cells, which causes the enlargement of pores facilitating the penetration of solvent into the cells that in turn helps escape active principles out into the solvent[49].

It is now clear that Soxhlet extraction is more efficient than percolation method in yielding higher content of capsaicinoids. Moreover, solvent and energy consumptions are less in Soxhlation compared to percolation method, which is highly advantageous because it decreases the processing cost of extraction. One advantage with the percolation method is that it is a cold extraction process which remains to be an important consideration while extracting thermolabile plant components. Microwave extraction and ultrasonication methods have several advantages over traditional Soxhlation method like less solvent is required, time taking for extraction is less and of course power consumption is also less. Moreover, in microwave irradiation method, acetone boils at a lower temperature than its actual boiling point making the extraction process safe and easy to run. Ultrasonication is even more advantageous than microwave irradiation method since it consumes less time to complete the extraction cycle with better yield of capsaicinoids. Ultrasonication is the quickest process among all these four methods of extraction.

From our above discussion, it is suggested that apart from Soxhlet method, ultrasonication followed by microwave techniques can be adopted in a laboratory set up for research/experimental studies as well as in small industrial scale to extract and isolate the bioactive pungent principles from *Bhut Jolokia* fruits with good yield. It is proposed that ultrasonication is the best method, since it is more efficient, convenient, and affordable than other extraction methods. Moreover, easy operation, less tediousness, and low processing cost are other important advantages of ultrasonication that makes it a suitable extraction technique to be proffered over other techniques for the extraction of capsaicinoids from *Bhut Jolokia*. However, well ventilation and skillful operation are required while handling such extraction processes, since capsaicinoids are highly irritating substances that may cause severe toxicity upon inhalation. Therefore, the extraction of capsicum must be carried out in a closed but well-ventilated environment, and all due precautions must be taken care while handling and processing such substances.

Effect of storage

Results shown in Fig. 2a indicate that the capsaicinoids content was enhanced by 3.673±0.21 % at the end of the 6th month, which was followed by a constant value, 3.673±0.18 % till the end of the 12th month. It accounts for approximately 50 % increase in the concentration of capsaicinoids content during the first half of the storage period. Similarly, weight loss was observed as 9.971±0.28 % (%, in g of dry weight of capsicum fruits) at the end of 6th month followed by a constant value (Fig. 2b).

During post-harvesting (storage) period, the enhancement of capsaicinoids content in capsicum pods is attributed to the induction of certain biochemical pathways of secondary plant metabolites under the influence of several external factors (presence of adequate moisture and absence of light) in stored environment. The biosynthesis of capsaicinoids gets accelerated through activation of phenylpropanoid pathway under hydric stress in a closed environment. Bioprecursor vanillylamine (substrate component) facilitates the formation of capsaicinoids with the help of an enzyme, called capsaicinoid synthase available in stored pods. The presence of adequate moisture is highly important for optimal accumulation of capsaicinoids in pods during the first few months of storage. After six months, accumulation of capsaicinoids declines due to dehydration of pods. The pungency level of capsicum gradually decreases with loss of water[50]. The capsaicinoids content of capsicum increases with the maturity of fruits until it reaches maximum followed by a gradual degradation[11]. The present study is consistent with several earlier literature reports on other capsicum species. For instance, fresh Jalapeno capsicum contains 1.89 % of capsaicinoids, whilst its dried pods contain around 2.63 %[27]. Contreras-Padilla and Yahia[28] observed that the decrease in capsaicinoids content in stored pods is a result of oxidative degradation, called peroxidation with the help of peroxidase enzyme. The enzyme remains high enough during the ripening of fruits and therefore, the concentration of capsaicinoids gradually declines after that period. Schweiggert *et al*[39] indicated that illumination restores the peroxidase enzyme and is mainly responsible for the loss of pungent principles of capsicum by oxidizing the vanillyl moiety of

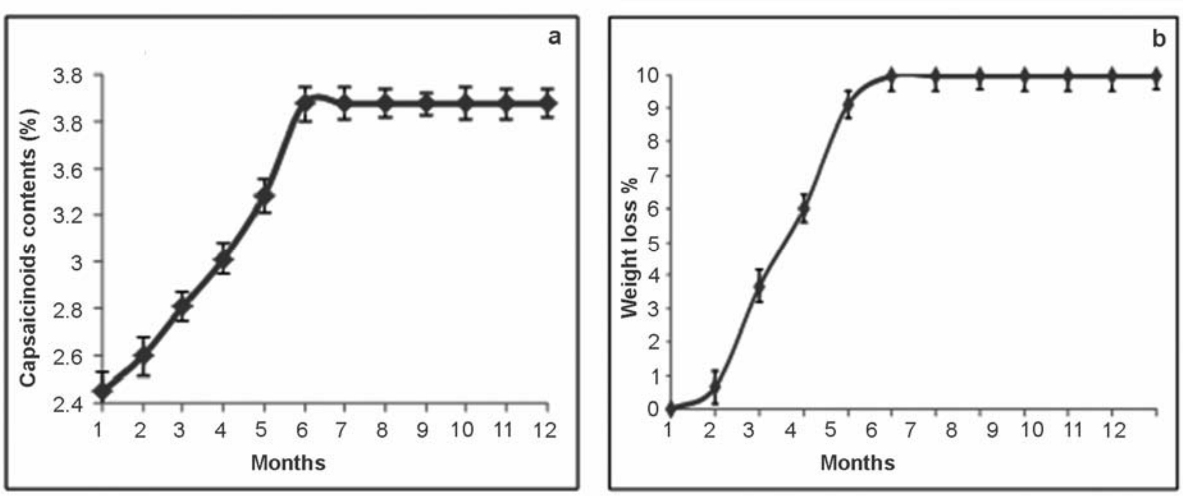

Fig. 2 — Graphs showing the effect of storage condition on (A) capsaicinoids content (%, in mg/g of capsaicin), and (B) weight loss (%, in g of dry weight of capsicum) of *Bhut Jolokia* fruits. Studies were performed in trplicate. Mean±SD values of three replicate observations (n=3) were used for plotting graphs.

capsaicinoids[39,51]. Instead, during long term storage in dark atmosphere dehydration stress (water loss) and presence of peroxides (R-O-O-R) (in store pods) cause degradation of the peroxidase enzyme and thus, significantly reduces the degradation activity of capsaicinoids[52,53]. From above discussion, it can be concluded that illumination induces photooxidation of capsaicinoids and reduces pungency of capsicum, whereas, dark atmosphere significantly prevents the oxidative degradation of capsaicinoids and restore the pungency level. Capsaicinoids content of capsicum may also be affected by the atmospheric gases like oxygen but not nitrogen during storage[39]. The phenolic hydroxyl group and carbon-carbon double bonds make capsaicinoids very much sensitive to oxidation, which render molecules degrade easily under such atmospheric stressed conditions. Natural capsaicinoids must therefore be protected from light, heat, moisture, and oxidizing agent like oxygen. The storage of capsicum fruits in dark avoiding exposure to atmospheric gases strongly renders prevention of degradation of capsaicinoids.

Conclusion

Keeping in view the dietary as well as medicinal importance of *Bhut Jolokia* fruits worldwide, proper cultivation, harvesting measures, and conservation strategies are required to be adopted with the objective to optimally utilize this valuable indigenous natural resource available in North-eastern region of India. Such strategies may help to improve the crop productivity and at the same time would also maintain the pungency level of *Bhut Jolokia*. Pungency is an indicator of capsicum's quality - It must be retained for this indigenous species of capsicum. To achieve it, further studies on chemistry and bioactivity profile of *Bhut Jolokia* fruits need to be done. To extract capsaicinoids in high content modern extraction methods like microwave and ultrasonication can be adopted in basic laboratory/research set ups as well as in small industrial, giving minimum preference to Soxhlet and percolation like traditional methods, since they are slow processes, time consuming and cost more. Further, traditional claim on pungency enhancement of *Bhut Jolokia* pods upon long term storage is validated. There is need for further research investigations and also a requirement to continue this practice on a commercial scale to meet the increasing demand of *Bhut Jolokia* fruits in the global market. Moreover, besides chemistry and bioactivity studies, toxicity investigation is inevitably required to unfold the science behind the unique pungency of *Bhut Jolokia* and its beneficial or toxic implications on human health.

Acknowledgement

Authors are very much thankful to Late Prof. (Dr.) A. K. Dolui for his novel ideas, generous help, and invaluable guidance towards successful completion of our research work, and also wish to dedicate this paper to his loving memory. KKS is grateful to Indian Council of Medical Research, New Delhi for providing Senior Research Fellowship (45/14/2011/BMS/TRM) to carry out the research work. The authors also thank Chillies Export House, Virudhanagar, India for providing the gift sample of capsaicin.

References

1 Meghvansia M K, Siddiquib S, Khan M H, Gupta V K, Vairale M G, Gogoi H K *et al*., Naga chilli: A potential source of capsaicinoids with broad-spectrum ethnopharmacological applications, *J Ethnopharmacol*, 2010, **132**, 1–14.

2 Sarwa K K, Kiran J, Sahu J, Rudrapal M and Debnath M, A short review on *Capsicum Chinese* Jacq., *J Herbal Med Toxicol*, 2012, **6**(2), 7-10.

3 Guinness Book of World Records, 2006, Hottest Spice, available from URL: http://www.guinessworldrecords.com, accessed 24th March 2012.

4 Mathur R, Dangi R S, Das S C and Malhotra R C, The hottest chilli variety in India, *Curr Sci*, 2000, **79**, 287–288.

5 Bosland P W and Baral J B, Bhut jolokia -The world's hottest known chile pepper is a putative naturally occurring interspecific hyrid, *HortSci*, 2007, **42**, 222-224.

6 Bhagowati R R and Changkija S, Genatic variability and traditional practices in Naga chilli landraces of Nagaland, *Asian Agri-History*, 2009, **13**, 171-180.

7 Molina-Torres J, García-Chávez A and Ramírez-Chávez E, Antimicrobial properties of alkamides present in flavouring plants traditionally used in Mesoamérica: affinin and capsaicin, *J Ethnopharmacol*, 1999, **64**, 241–248.

8 Sarwa K K, Mazumder B, Rudrapal M and Verma V K, Potential of capsaicin-loaded transfersomes in arthritic rats, *Drug Deliv*, 2015, **22**(5), 638–646.

9 Sarwa K K, Mazumder B, Rudrapal M, Debnath M, Kumar A, Verma V K *et al*, Capsaicinoids content of some indigenous capsicum varieties of Assam, India, *J Nat Sci Res*, 2013, **3**(4), 112-116.

10 Hoffman P G, Lego M C and Galetto W G, Separation and quantitation of red pepper major heat principles by reverse-phase high pressure liquid chromatography, *J Agric Food Chem*, 1983, **31**, 1326–1330.

11 Iwai K, Suzuki T and Fujiwake H, Formation and accumulation of pungent principle of hot pepper fruits, capsaicin and its analogues, in *Capsicum annuum* var. *annuum* cv. Karayatsubusa at different growth stages after flowering, *Agric Biol Chem*, 1979, **43**, 2493–2498.

12 Mozsik G, Past T, Salam O M A, Kuzma M and Perjesi P, Interdisciplinary review for correlation between the plant origin capsaicinoids, non-steroidal antiinflammatory drugs,

gastrointestinal mucosal damage and prevention in animals and human beings, *Inflammopharmacol*, 2009, **17**, 113-150.

13 Kirschbaum-Titze P, Mueller-Seitz E and Petz M, Pungency in paprika (*Capsicum annuum*): Heterogeneity of capsaicinoid content in individual fruits from one plant, *J Agric Food Chem*, 2002, **50**, 1264–1266.

14 Rahman M J and Inden H, Effect of nutrient solution and temperature on capsaicin content and yield contributing characteristics in six sweet pepper (*Capsicum annuum* L.) cultivars, *J Food Agric Environ,* 2012, **10**(1), 524-529.

15 Govindarajan V S, Rajalakshmi D and Chand N, Critical Review: Capsicum – Production, technology, chemistry, and quality-Part IV: Evaluation of quality, *Food Sci Nutr*, 1987, **25**, 185–282.

16 Masood A, Dogra J V V and Jha A K, The influence of colouring and pungent agents of red chilli (*Capsicum annuum*) on growth and aflatoxin production by *Aspergillus flavus, Lett Appl Microbiol*, 1994, **18**, 184–186.

17 Webster L R, Peppin J F, Murphy F T, Tobias J K and Vanhove G F, Tolerability of NGX-4010, a capsaicin 8% patch, in conjunction with three topical anesthetic formulations for the treatment of neuropathic pain, *J Pain Res*, 2012, **5**, 7–13.

18 Sarwa K K, Rudrapal M and Mazumder B, Topical ethosomal capsaicin attenuates edema and nociception in arthritic rats, *Drug Deliv*, 2015, **22**(8), 1043–1052.

19 Macho A, Lucena C, Sancho R, Daddario N, Minassi A, Munoz E *et al*., Nonpungent capsaicinoids from sweet pepper synthesis and evaluation of the chemopreventive and anticancer potential, *Eur J Nutr*, 2003, **42**, 2-9.

20 Materska M and Perucka I, Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.), *J Agric Food Chem*, 2005, **53**, 1750-1756.

21 Liu Y and Nair M G, Capsaicinoids in the hottest pepper Bhut Jolokia and its antioxidant and antiinflammatory activities, *Nat Prod Commun*, 2010, **5**, 91-94.

22 Schweiggert U, Mix K, Schieber A and Carle R, An innovative process for the production of spices through immediate thermal treatment of the plant material, *Innov Food Sci Emerg Tech*, 2005, **6**, 143–15.

23 Li H, Pordesimo L O, Igathinathane C and Vinyard B, Physical property effects on drying of chile peppers, *Int J Food Prop*, 2009, **12**, 316-330.

24 Sung Y, Chang Y Y and Ting N L, Capsaicin biosynthesis in water-stressed hot pepper fruits, *Bot Bull Acad Sin*, 2005, **46**, 35-42.

25 Ochoa-Alejo N, and Ramírez-Malagón R, *In vitro* chili pepper biotechnology, *In Vitro Cell Dev Biol Plant,* 2001, **37**, 701-729.

26 Estrada B, Pomar F, Díaz J, Merino F and Bernal M A, Pungency level in fruits of the Padrón pepper with different water supply, *Sci Hortic*, 1999, **81**, 385–396.

27 Castillo E, Torres-Gavilán A, Severiano P, Arturo N and López-Munguía A, Lipase-catalyzed synthesis of pungent capsaicin analogues, *Food Chem*, 2007, **100**, 1202-1208.

28 Contreras-Padilla M and Yahia E M, Changes in capsaicinoids during development, maturation, and senescence of chile peppers and relation with peroxidase activity, *J Agric Food Chem*, 1998, **46**, 2075-2079.

29 Castillo E, Lopez-Gonzalez I, De Regil-Hernandez R, Reyes-Duarte D, Sánchez-Herrera D, Lopez-Munguia A *et al*, Enzymatic synthesis of capsaicin analogs and their effect on the T-type Ca^{2+} channels, *Biochem Biophys Res Comm*, 2007, **356**, 424-430.

30 Kaga H, Miura M and Orito KA, Facile procedure for synthesis of capsaicin, *J Org Chem*, 1989, **54**, 3477-3478.

31 Gbolade A A, Omobuwajo O R and Soremekun R O, Evaluation of the quality of Nigerian chillies for pharmaceutical formulations, *J Pharm Biomed Anal*, 1997, **15**, 545-548.

32 Gnayfeed M H, Daood H G, Ille´s V and Biacs P A, Supercritical CO_2 and subcritical propane extraction of pungent paprika and quantification of carotenoids, tocopherols, and sapsaicinoids, *J Agric Food Chem*, 2001, **49**, 2761-2766.

33 Sato K, Sakamoto S and Goda Y, Direct connection of supercritical fluid extraction and supercritical fluid chromatography as a rapid quantitative method for capsaicinoids in placentas of Capsicum, *J Agric Food Chem*, 1999, **47**, 4665-4668.

34 Santamarı´a R I, Reyes-Duarte M D, Ba´rzana E, Ferna´ndo D, Gama F M. Mota M *et al*, Selective enzyme-mediated extraction of capsaicinoids and carotenoids from chili Guajillo Puya (*Capsicum annuum* L.) using ethanol as solvent, *J Agric Food Chem*, 2000, **48**, 3063-3067.

35 Salgado-Roman M, Botello-Alvarez E, Rico-MartiInez R, Jimenez-Islas H, Cardenas-Manriquez M and Navarette-Balanos J L, Enzymatic Treatment To Improve Extraction of Capsaicinoids and Carotenoids from Chili (*Capsicum annuum*) Fruits, *J Agric Food Chem*, 2008, **56**, 10012–10018.

36 Ganzler K, Microwave extraction-A novel simple preparation method for chromatography, *J Chromatogr*, 1986, **371**, 299-306.

37 Ganzler K and Szinai I, Effective sample preparation method for extracting biologically active compounds from different matrices by a microwave technique, *J Chromatogr*, 1990, **520**, 257-262.

38 Hemwimol S, Pavasan P and Shotipruk A, Ultrasound-assiated extraction of anthraquinones from roots of *Morinda citrifolia*, *Ultrason Sonochem*, 2006, **13**, 543-548.

39 Schweiggert U, Schieber A and Carle R, Effects of blanching and storage on capsaicinoids stability and peroxidase activity of hot chili pepper (*Capsicum frutescens* L), *Innov Food Sci Emerg Tech*, 2006, **7**, 217-224.

40 Sarwa K K, Rudrapal M and Debnath M, Extraction of Green Tea Leaves: The use of Different Methods, their Optimization and Comparative Evaluation, *Biosci Biotech Res Asia*, 2013, **10**(1), 383-386.

41 Medziak I and Waksmundski A, Selection of composition and concentration of the mobile phase for separation of phenol derivatives by TLC, *Chem Anal*, 1981, **28**, 251-257.

42 Sankarikutty B, Sumathikutty M A and Narayanan C S, Standardization of extraction of pungency from whole chilli (Capsicum) for estimation of capsaicin, *J Food Sci Technol*, 1978, **15**, 126-127.

43 Maillard M, Giampaoli P and Richard H M J, Analysis of eleven capsaicinoids by reversed-phase high performance liquid chromatography, *Flav Frag J*, 1997, **12**, 409-413.

44 Sadasivam S and Manikam A, *Biochemical Methods for Agricultural Sciences* (Wiley Eastern Ltd., New Delhi), 1992, 112-126.

45 Amruthraj N J, Raj J P P and Lebel L A, Polar aprotic extraction of capsaicinoids from *Capsicum chinense*, *Bhut Jolokia* fruit for antimicrobial activity, *Int J Biol Pharm Res*, 2013, **4**(12), 959-964.

46 Attuquayefio V and Buckle K, Rapid sample preparation method for HPLC analysis of capsaicinoids in Capsicum fruits and oleoresins, *J Agric Food Chem*, 1987, **35**, 777-779.

47 Alonso-Salces R M, Korta E, Barranco A, Berrueta L A, Gallo B and Vicente F, Determination of polyphenolic profiles of Basque cider apple varieties using accelerated solvent extraction, *J Agric Food Chem*, 2001, **49**, 3761-3767.

48 Sharma U K, Sharma K, Sharma N, Sharma A, Singh H P and Sinha A K, Microwave-assisted efficient extraction of different parts of *Hippophae rhamnoides* for the comparative quantification of its phenolic constituents by reverse-phase high-performance liquid chromatography (RP-HPLC), *J Agric Food Chem*, 2008, **56**, 374–379.

49 Hemwimol S, Pavasant P and Shotipruk A, Ultrasound-assisted extraction of anthraquinones from roots of *Morinda citrifolia*, *Ultrason Sonochem*, 2006, **13**, 543–548.

50 Lownds N K, Banaras M and Bosland P W, Relationships between postharvest water loss and physical properties of pepper fruit (*Capsicum annuum* L.), *Hort Sci*, 1993, **28**, 1182–1184.

51 Zapata J M, Calderón A A, Muñoz R and Barcelo A R, Oxidation of natural hydroxybenzoic acids by grapevine peroxidases: Kinetics characteristics and substrate specificity, *Am J Enol Vitic*, 1992, **43**, 134–138.

52 Bernal M A, Calderon A A, Pedreno M A, Munoz R, Barcelo A R and Caceres F M, Capsaicin oxidation by peroxidase from *Capsicum annuum* (var. *annuum*) fruits, *J Agric Food Chem*, 1983, **41**, 1041-1044.

53 Bernal M A, Calderon A A, Pedreno M A, Munoz, R, Barcelo A R and Caceres F M, Dihydrocapsaicin oxidation by *Capsicum annuum* (var. annuum) peroxidase, *J Food Sci*, 1983, **58**, 611-613.