ORIGINAL ARTICLE

Phytochemical properties, antioxidant activity, and color content of different colored fruits of *Capsicum* species cultivars

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Abstract

Introduction: Peppers have been recognized as an excellent source of antioxidant constituents with diverse medicinal functions. Maturation stages, genotypes, and different drying conditions lead to changes in phytochemicals content and antioxidant activity of peppers. It is, therefore, a vital importance to study the changes in the content of color, phytochemicals, capsaicinoids, and antioxidants level of peppers, as influenced by different genotypes, their maturity stages, and drying processes. **Materials and Methods:** In the present work, efforts were made to evaluate the color, capsaicinoids content, phenolics, and flavonoids contents as well as antioxidant capacity in traditionally dried mature fruits and in oven-dried different colored fruits of *Capsicum* species, namely, *Capsicum annuum* L. (cvs "Meiteimorok" and "Sirarakhong morok"), *Capsicum frutescens* L. (cvs "Uchithi" and "Mashingkha"), and *Capsicum chinense* Jacq. (cvs "Umorok" and "Chiengpi"). **Results and Discussion:** The amount of total phenolic compounds, flavonoids, capsaicinoids, antioxidant capacity, and extractable color measured in the units of the American Spice Trade Association varied significantly in the different genotypes and also under different drying conditions and in differently colored stages. Traditionally dried samples, in general, showed higher content of phenolics, flavonoids, and capsaicinoids in all the cultivars whereas the color content and antioxidant activity were found to be higher in oven-dried samples. With maturation, most of the cultivars showed an increasing trend with regard to color content and the phenolics, flavonoids, and capsaicinoids contents. However, the antioxidant activity decreased with increased maturity stages of the samples. **Conclusion:** Thus, this study signifies the role of genotype, maturity stage, and drying methods in determining the color content, antioxidant activity, and bioactive constituents of peppers.

Key words: *Capsicum*, maturation stages, drying methods, antioxidant activity, biochemical analysis

Key Messages: The present study shows that chili samples analyzed are a good source of antioxidants and secondary metabolites, and the bioactive constituents of peppers are significantly determined by the maturity stage, genotype, and drying methods. Therefore, maturity stage, genotype, and drying methods are the important parameters to be considered for optimum bioactive constituents

INTRODUCTION

The genus *Capsicum* comprises more than 200 varieties of peppers with economical value and most of the widely distributed varieties belong to three common species: *Capsicum annum*, *Capsicum frutescens,* and *Capsicum chinense*.[1-3] *Capsicum* cultivars, which are immensely valued for their sensory attributes of color, pungency, and flavor, have been identified as potential vegetables in human nutrition in many parts of the world.[4] Hence, they have been employed in food industry as spices, coloring and flavoring agents in sauces, snacks, candies, soups, processed meats, soft drinks, and alcoholic beverages.[5] Chili peppers are considered one of the richest sources of carotenoids, Vitamin C, and phenolic compounds such as phenolics acids, flavonoids,

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Received: 03-10-2018
Revised: 04-09-2021
Accepted: 12-10-2021

hydroxycinnamates, and flavones, which have indeed caused great interest due to their antioxidant activity and thus surpassed that of many other antioxidants.[6-9] Phenolic compounds contribute to fruit sensory and nutritive quality in terms of modifying color, taste, aroma, and flavor as well as provide potential health-beneficial effects.[10] The pungency of *Capsicum* fruit is due to a group of compounds called capsaicinoids while their attractive red color is due to the profuse synthesis of carotenoid pigments such as capsanthin, capsorubin, and cryptocapsin, which are exclusively present in hot pepper varieties in different amounts and have been shown to be effective free radical scavengers.[11,12]

The compositional quality and content of phytochemicals in plant material are found to be influenced by numerous factors including climatic conditions, ripening time, genotype, and cultivation techniques. Among these factors, the maturity stage of fruits and vegetables is an important factor since, during fruit ripening, several biochemical, physiological, and structural modifications occur that determine the attributes of fruit quality and composition.[2] Different cultivars/genotypes of the same fruits and vegetables, even in peppers, exhibited wide range of variations in their overall phytochemical content, color content, and capsaicinoids content in response to various factors such as biotic and abiotic stresses, genotypes, maturity stages, and different processing conditions.[3,13-18] All these factors also have a profound effect on the level of antioxidant activity of different peppers.[7,20-23]

In general, peppers are commonly dried by two methods: Traditional method which involves direct sun exposure or drying with wood smoke[25-27] and mechanical heat method which involves drying in oven and microwaves.[28] Even though traditional drying methods prove to be more economical, it requires longer time and does not have controlled temperature. Moreover, it causes a major loss of colorant texture quality and bioactive compounds because of enzymatic activities and contamination by fungi, bird, and insect activities.[25,29-31] On the contrary, oven-drying methods offer more control over the temperature, product quality, and loss of color as well as degradation of bioactive compounds present in peppers.[28,32,33] Variability of color content has been shown to be dependent mainly on drying conditions, in which degradation rate increases with the increase in drying temperature.[34,35] Thus, proper controlled temperature for drying peppers is a prerequisite for preserving its color as well as bioactive compounds present in it. An efficient drying technique increases the shelf life of peppers as well as enhances their aroma and appearance.[24]

The knowledge of the changes which occur during maturation stages and different processing conditions are of immense significance to be studied from both dietary and nutritional points of view. Many publications have reported difference in bioactive content with different processing conditions, maturity stages and genotypes, although contradictory results were obtained probably due to different varietal factors. It is, therefore, a vital importance to study the changes in the content of color, phytochemicals, capsaicinoids, and antioxidants level of peppers, as influenced by different genotypes, their maturity stages, and drying processes. Moreover, there are numerous reports available on phytochemical studies and effect of many drying techniques on the bioactive content and quality of different *Capsicum* species.[29,34-42] Therefore, the present work was undertaken to evaluate the color, capsaicinoids, phenolics, and flavonoids content as well as the antioxidant capacity of oven-dried differently colored fruits and traditionally dried mature fruits of six chili cultivars belonging to three species of *Capsicum*: *Capsicum annuum* L. (cvs "Meiteimorok" and "Sirarakhong morok"), *C. frutescens* L. (cvs "Uchithi" and "Mashingkha"), and *C. chinense* Jacq. (cvs "Umorok" and "Chiengpi"). These six chili cultivars form economically important food crops of Manipur[3] and studying the effect of drying conditions and maturation stages on bioactive content and antioxidant capacity of the cultivars will help in characterization of the food value of these cultivars.

MATERIALS AND METHODS

Sampling Procedures

Traditionally dried chili and fresh fruits of the six chili samples ("Meiteimorok," "Sirarakhong morok," "Uchithi," "Mashingkha," "Umorok," and "Chiengpi") used in the present study were collected from local market and cultivation fields of Manipur [Figure 1]. The different colored fresh fruits at different maturation stages were collected and oven-dried at 60°C for 12 h. Dried samples were ground into finely powdered form and stored in refrigerator at 4°C until further analysis.

Color Content

The American Spice Trade Association (ASTA) color value was determined according to the Association of Official Analytical Chemists International (2002)[43] method with slight modifications. One gram of dried chili samples was extracted with 10 ml of absolute acetone, incubated at 60°C for 4 h using water bath with constant shaking. Then, the extract was diluted with 1/5 acetone. The absorbance of the extract was measured against acetone at 460 nm by spectrophotometer. Total extractable color content is given in internationally recognized units for extractable color, ASTA units.

Extraction of Samples and Quantitative Estimation of Secondary Metabolites

One gram of powdered chili samples was extracted with 10 ml of 90% methanol and incubated at room temperature for 4 h swirling manually every hour. After cooling at room

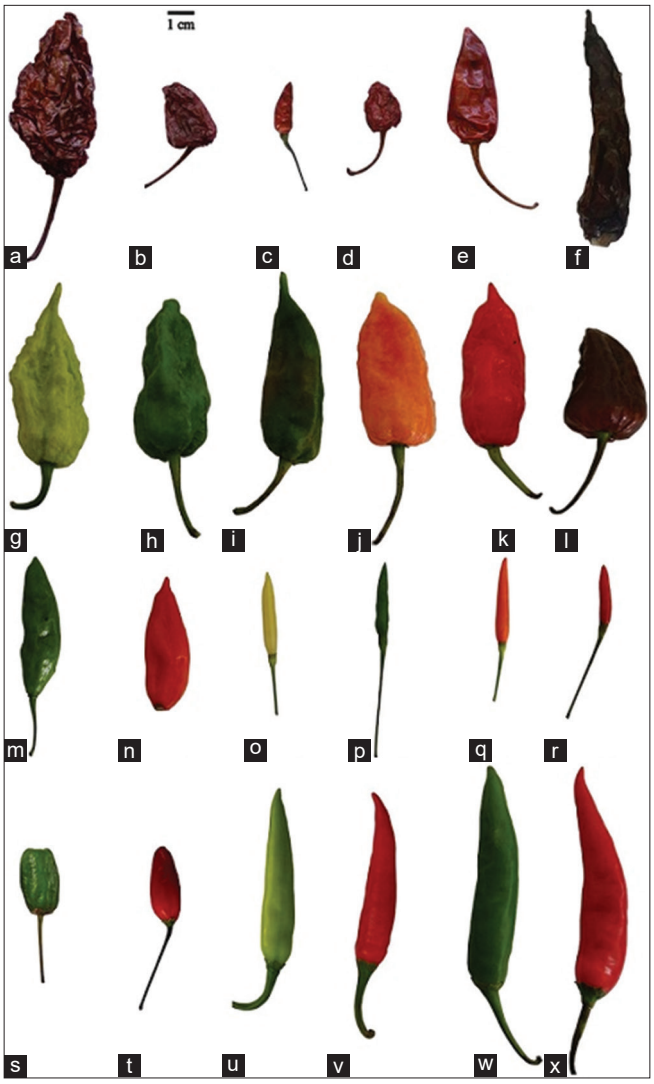

Figure 1: Chili samples belonging to six species of *Capsicum*: Traditionally dried (a) "Umorok," (b) "Chiengpi," (c) "Uchithi," (d) "Mashingkha," (e) "Meitei morok," (f) "Sirarakhong morok," and fresh samples used for oven drying of (g) Light green "Umorok," (h) green "Umorok," (i) dark green "Umorok," (j) orange "Umorok," (k) red "Umorok," (l) brown "Umorok," (m) green "Chiengpi," (n) red "Chiengpi," (o) yellow "Uchithi," (p) green "Uchithi," (q) orange "Uchithi," (r) red "Uchithi," (s) green "Mashingkha," (t) red "Mashingkha," (u) green "Meiteimorok," (v) red "Meiteimorok," (w) green "Sirarakhong morok," and (x) red "Sirarakhong morok"

temperature, it was centrifuged at 7500 rpm for 15 min. The supernatant was collected and used for the determination of antioxidant activity following the method of Shimada *et al*.[44] using 1, 1-diphenyl-2-picrylhydracyl (DPPH), phenolic content following the Folin–Ciocalteu method of Singleton *et al*.,[45] flavonoid content following aluminum chloride method of Jagadish *et al*.,[46] and capsaicinoids content following the method of Salgado-Roman *et al*.[47] The scavenging activity of the sample corresponded to the intensity of quenching of DPPH and the results were expressed as percentage of inhibition and the pungency of the chili samples are expressed in Scoville Heat Units (SHUs).

Statistical Analysis

All experiments were repeated thrice, each consisting of three replicates and data were analyzed using one-way analysis of variance (ANOVA, $P < 0.05$). The significant differences among the means were determined by *Duncan's* multiple range test.

RESULTS AND DISCUSSION

The results of quantitative estimation of color, antioxidant activity, phenol, flavonoids, and capsaicinoids content of all the analyzed samples are given in Table 1. The extractable ASTA color of the chili samples varied depending on the genotype, drying methods, and maturity stages [Table 1]. The ASTA values for the analyzed chili samples ranged from 51.88 ASTA units in oven-dried green-colored "Meitei morok" to 170.63 ASTA units in oven-dried red-colored "Sirarakhong morok." The ASTA values in oven-dried samples with 170.63 ASTA units/g dry weight (DW) in red-colored "Sirarakhong morok" are higher than the traditionally dried "Sirarakhong morok" (151.94 units/g DW) indicating the preservation of color during oven drying. Earlier, similar enhancement of color content in oven-dried samples of *C. annuum* L. was reported by Vega-Galvez.[48] Garcia *et al*.[37] observed an increasing trend (91–150 ASTA units) in the color content during maturity in majority of *Capsicum* cultivars and a similar trend was also observed in the present study in which among all the oven-dried samples, the matured red color peppers showed the highest ASTA value followed by brown, orange, yellow, dark green, green, and light green colored ones.

Genotype and maturity stages have been shown to play a crucial role in determining the antioxidant activity of peppers.[49,50] In the present study also, the percentage inhibition determined by DPPH free radical scavenging assay varied in the range of 23.42% in traditionally dried samples of "Mashingkha" to 86.80% in oven-dried light green-colored "Umorok" samples [Table 1]. The oven-dried "Umorok" samples at light green- and green-colored stages of maturity exhibited the highest % inhibition with 86.80% and 85.37%, respectively. High temperatures during drying have been shown to have no negative effect on the antioxidant activity of peppers,[42,48,51] and similarly, the oven-dried samples exhibited higher antioxidant activity compared to the traditionally dried samples in the present study also [Table 1]. Among the differently colored samples, peppers sampled at green-colored stages showed higher antioxidant activity in all the genotypes except in "Uchithi," where the yellow-colored fruits showed higher antioxidant activity. Similar observation of higher antioxidant activity at green stage has been reported earlier.[52,53] However, other studies have reported an increase in antioxidant activity with maturation from green to red stages, regardless of genotypes.[4,7,39,54]

Table 1: Color, phenols, flavonoids, antioxidant activity, and capsaicinoids content of different samples of six chili cultivars belonging to three species of *Capsicum: Capsicum chinense* Jacq. (cvs "Umorok" and "Chiengpi"), *Capsicum frutescens* L. (cvs "Uchithi" and "Mashingkha"), and *Capsicum annuum* L. (cvs "Meiteimorok" and "Sirarakhong morok")

Samples	Color content (ASTA unit)	Phenols content (mg g^{-1})	Flavonoids content (mg g^{-1})	% inhibition (DPPH)	Capsaicin (mg g^{-1})	Dihydrocapsaicin (mg g^{-1})
Traditionally dried						
"Umorok"	101.94±3.81^{k}	27.35±0.37^{g}	6.22±0.16^{e}	40.14±0.12^{l}	16.00±0.13^{b}	9.01±0.05^{b}
"Chiengpi"	118.74±2.95^{gh}	14.84±0.26^{m}	12.39±0.09^{b}	59.25±0.16^{j}	9.63±0.12^{f}	4.00±0.10^{h}
"Uchithi"	116.53±2.93^{hi}	43.50±0.12^{b}	10.97±0.13^{c}	72.38±0.13^{f}	8.01±0.14^{g}	4.00±0.14^{h}
"Mashingkha"	113.32±2.85^{ij}	31.06±0.11^{e}	12.78±0.11^{a}	23.42±0.24^{r}	10.01±0.23^{e}	5.20±0.11^{f}
"Meiteimorok'	126.54±3.78^{e}	23.25±0.28^{j}	5.91±0.15^{e}	64.35±0.14^{h}	4.21±0.10^{m}	2.50±0.10^{j}
"Sirarakhong morok'	151.94±2.76^{b}	20.89±0.32^{k}	10.18±0.15^{d}	29.00±0.23^{o}	4.81±0.13^{l}	3.10±0.11^{i}
Oven dried						
Light green "Umorok'	71.53±2.87^{m}	1.85±0.15^{t}	0.30±0.05^{n}	86.80±0.13^{a}	15.05±0.11^{d}	8.00±0.10^{d}
Green "Umorok"	72.03±3.92^{m}	2.59±0.13^{s}	0.39±0.10^{n}	84.37±0.15^{b}	16.00±0.12^{b}	9.00±0.07^{b}
Dark green "Umorok"	72.59±4.04^{m}	1.74±0.09^{t}	0.31±0.04^{n}	80.40±0.43^{d}	16.32±0.09^{a}	9.20±0.09^{a}
Orange "Umorok"	97.84±2.03^{k}	2.83±0.02^{s}	0.53±0.09^{m}	59.53±0.31^{j}	15.40±0.12^{c}	8.40±0.10^{c}
Red "Umorok"	112.39±2.0^{ij}	4.84±0.08^{r}	0.54±0.07^{m}	26.55±0.31^{q}	15.50±0.13^{c}	7.55±0.02^{e}
Brown "Umorok"	99.31±2.06^{k}	1.86±0.19^{t}	0.51±0.05^{m}	25.81±0.15^{q}	16.01±0.16^{b}	9.05±0.12^{b}
Green "Chiengpi"	114.46±2.10^{j}	14.77±0.13^{m}	3.75±0.12^{g}	31.01±0.25^{n}	7.12±0.11^{hi}	4.00±0.10^{h}
Red "Chiengpi"	124.33±2.06e^{f}	24.61±0.23^{i}	4.87±0.16^{f}	64.06±0.36^{h}	7.30±0.16^{h}	4.05±0.07^{h}
Green "Uchithi"	113.30±2.02^{ij}	9.17±0.16^{q}	0.24±0.05^{n}	75.70±0.24^{e}	6.68±0.16^{j}	3.10±0.12^{i}
Yellow "Uchithi"	81.90±3.03^{l}	45.85±0.11^{a}	1.05±0.17^{l}	82.89±0.25^{c}	6.70±0.12^{j}	3.15±0.11^{i}
Orange "Uchithi"	112.69±2.04^{ij}	33.86±0.11^{d}	1.33±0.14^{kl}	60.23±0.36^{i}	7.00±0.15^{i}	3.09±0.04^{i}
Red "Uchithi"	133.58±2.14^{d}	11.75±0.10^{p}	2.78±0.14^{h}	74.84±0.39^{e}	8.00±0.20^{g}	4.00±0.12^{h}
Green "Mashingkha"	110.93±3.04^{j}	13.50±0.13^{o}	1.56±0.17^{jk}	68.51±0.32^{g}	6.12±0.17^{k}	3.90±0.09^{h}
Red "Mashingkha"	121.97±2.05^{fg}	40.79±0.13^{c}	2.72±0.15^{h}	47.27±0.14^{k}	8.10±0.10^{g}	4.65±0.13^{g}
Green "Meiteimorok"	51.88±3.02^{o}	17.51±0.10^{l}	1.80±0.13^{ij}	39.22±0.11^{m}	3.90±0.05^{n}	2.02±0.09^{k}
Red "Meiteimorok"	145.71±2.05^{c}	25.23±0.11^{h}	1.99±0.13^{i}	31.25±0.11^{n}	3.98±0.11^{mn}	2.10±0.08^{k}
Green "Sirarakhong morok"	61.91±1.03^{n}	14.26±0.15^{n}	0.09±0.02^{p}	29.61±0.31^{o}	4.05±0.06^{mn}	2.70±0.11^{j}
Red "Sirarakhong morok"	170.63±2.08^{a}	28.08±0.10^{f}	0.19±0.05^{o}	27.34±0.23^{p}	4.25±0.15^{m}	3.00±0.07^{i}

Data represents mean±SD. Duncan's comparisons are significant when letters are different within columns (ANOVA, $P < 0.05$)

The total soluble phenolic content was determined by the Folin–Ciocalteu assay and the total phenol content of the chili samples varied depending on the cultivar, drying method, and differently colored stages of the samples. The total polyphenol content varied in all analyzed samples and ranged from 1.85 mg/g DW in oven-dried light-green colored "Umorok" to 45.85 mg/g DW in oven-dried yellow-colored "Uchithi," showing wide variation among the samples. The phenol content observed in the samples is considerably higher compared to those of other cultivars reported earlier.[38-40,48]

Vega-Galviz *et al*.[52] have previously reported that high drying temperature promotes phenols content in peppers. Similarly, in all the samples analyzed, higher phenol contents were recorded in oven-dried samples compared to traditionally dried chili samples except in "Umorok" samples, in which the traditionally dried samples showed higher content than oven-dried samples. The availability of precursor of phenolic molecules by non-enzymatic interconversion between phenolic molecules might be the reason for the formation of phenolic compounds at high temperature.[55] In general,

the total phenol content increased with maturity, regardless of genotypes, as reported in the previous studies[7,39,56,57] except in a few studies which reported the decrease in total phenol content during maturation from green to red.[2,58] Flavonoids content also varied greatly among the pepper samples analyzed and the total content ranged from 0.09 mg/g DW in oven-dried green-colored "Sirarakhong morok" to 12.78 mg/g DW traditionally dried "Mashingkha" [Table 1 and Figure 2]. The traditionally dried samples had higher flavonoids content than the oven-dried ones and the flavonoids content increased with maturation from green to red stage in all the analyzed genotypes. Similar results were observed by Materska and Perucka,[36] in which red peppers produced higher flavonoids than green samples. However, some previous studies reported the decrease in flavonoids content with maturation,[2,7,58] which is in contradiction with the present finding.

Environmental conditions, processing conditions, and maturity stages of fruits are considered to have an impact on capsaicinoids levels in peppers.[34,35] The content of capsaicin and dihydrocapsaicin in the analyzed samples also varied ranging from 3.90 mg/g DW and 2.02 mg/g DW capsaicin and dihydrocapsaicin, respectively, corresponding to 94,720 SHU in oven-dried green-colored "Meiteimorok" to 16.32 mg/g DW and 9.20 mg/g DW capsaicin and dihydrocapsaicin, respectively, corresponding to 408,320 SHU in dark green-colored oven-dried "Umorok" [Table 1], which is similar to the earlier reports.[3] Higher capsaicin and dihydrocapsaicin contents were observed in traditionally dried mature red samples than oven-dried red samples. Such decrease of capsaicin and dihydrocapsaicin content during oven drying with high drying temperature has been reported by Wang and Lin[57] and it might be due to enzymatic or chemical degradation of the heat sensitive capsaicinoids. Among the different colored samples at different maturation stages for "Umorok," the dark green-colored stage exhibited the highest capsaicinoids content and showed a decreasing trend in capsaicinoids content with advancing maturity. These variations of capsaicinoids content with maturity stages have been reported by earlier workers[2,39,58] and may be attributed to inherent variation in the level of peroxidase enzymes of the peppers.[34]

Thus, the present study signifies that maturity stage, genotype, and drying methods are important factors that play a crucial role in determining the color content, antioxidant activity, and bioactive constituents of peppers. In general, the chili samples analyzed are a good source of natural antioxidants and secondary metabolites and consumption of these fruits will add more nutritional benefits to foods.

CONCLUSION

In the present study, different drying conditions and maturity stages of differently colored chili peppers exhibited different amount of total phenolic compounds, flavonoids, capsaicinoids, antioxidant capacity, and extractable color contents. Although the secondary metabolites content and antioxidant activities of the studied Capsicum cultivars varied with maturity stage, genotype, and drying methods used, most of the cultivars showed rich content of antioxidants and secondary metabolites and their consumption may add more nutritional benefits to foods.

ACKNOWLEDGMENT

The authors are thankful to the Department of Biotechnology (DBT), Government of India, New Delhi, for financial support (*vide* No. BT/PR6387/AGII/106/876/2012).

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Source of Support: Department of Biotechnology, Government of India, New Delhi (*vide* No. BT/PR6387/AGII/106/876/2012). **Conflicts of Interest:** None declared.