Changes in Dry Matter, Oleoresin and Bioactive Components during Ripening of Different Chilli Pepper Cultivars of Nepal

U. Subedi[1@], R. Karki[1], P. Ojha[1] & S.L. Shrestha[2]

Abstract

The interest in the consumption of chilli pepper fruits (Capsicum annum L.) is, to large extent, due to its content of bioactive compounds and their importance as dietary antioxidants. In the present study, the effects of harvest time (based on maturity stage) on changes in polyphenol, flavonoid, ascorbic acid, beta carotene, total carotenoids, antioxidant activity, oleoresin and dry matter content in six different chilli pepper cultivars viz.; 'HRD-CHI-009', 'HRD-CHI-010', 'HRD-CHI-012', 'HRD-CHI-014', 'Akabare', and 'Jire' grown in Nepal were investigated. The results showed that concentration of bio-active components varied significantly among chilli pepper cultivars at both matured and fully ripened stages. With advanced maturation ascorbic acid, beta carotene, total carotenoids, and antioxidant content increased; however, polyphenol and flavonoid content decreased. Furthermore, oleoresin and dry matter content also increased with advanced maturation. Among the six different chilli cultivars used in this study, Akabare cultivar was the best cultivar from the aspect of flavonoid, polyphenol, beta carotene, and total carotenoids contents while, HRD-CHI-012 and HRD-CHI-009 were the best cultivars for ascorbic acid and dry matter contents, respectively.

Keywords: *Antioxidants, Chilli pepper, Flavonoids, Maturity stages, Polyphenol*

Introduction

Chilli pepper *(Capsicum annum L.)* is an important horticultural crop of major economic significance globally, imparting flavor, aroma, and color to foods (Caporaso et al. 2013). Since longtime hot chilli pepper fruit has been known all over the world as a delicious spice with a characteristic smell and taste used for preparing spicy sauces, Mexican and Asian cuisines (Henderson, 1992). The typical spiciness of chilli pepper fruit is due to the group of alkaloids called capsaicinoids where twelve different compounds have been identified (Kobata, 1998) but capsaicin and dihydrocapsaicin are responsible for about 90% of the spiciness (Iwai, 1979). In addition to its economic importance chilli pepper are equally important from health aspect because it is an excellent source of ascorbic acid, natural colors and different antioxidant compounds (Howard et al. 2000). During ripening stage, red, yellow and orange colors are developed in chilli pepper due to carotenoid pigments. More than 30 other different pigments have been identified in chilli pepper fruits (Matus et al. 1991). The ascorbic acid, natural pigments, and other antioxidant compounds found in chilli pepper are important health-promoting factors; adequate intake of these compounds in daily food intake can minimize the risk of different diseases including cancer and cardiovascular diseases (Bramley, 2000).

Ascorbic acid/Vitamin C, an important compound present in chilli pepper, chelates heavy metal ions (Namiki, 1990), reacts with singlet oxygen and other free radicals, and suppresses peroxidation, reducing the risk of arteriosclerosis, cardiovascular diseases, and some forms of cancer (Navarroet al.2006). Similarly phenolic compounds like polyphenol and flavonoids also reduce the risk of cardiovascular disease, strokes and certain forms of cancer (Prior

[1] Food Research Division, National Agricultural Research Institute, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal

[2] Horticulture Research Division, National Agricultural Research Institute, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal

[@] Corresponding author: uj.subedi@gmail.com ; uj.subedi@narc.gov.np

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and Cao, 2000). Levels of bioactive compounds can vary by genotype and maturity stage (Patthamakanokporn et al. 2008). Maturity stage is an important factor affecting antioxidant capacity and phytochemical content of chilli peppers (Patthamakanokpornet al. 2008).

Different international studies have shown that chilli pepper contains a wide range of health-promoting phytochemicals, but several chilli pepper species available in Nepal haven't been assessed for these important compounds. In this study, we assessed the effect of different maturity stages on dry matter, oleoresin, vitamin C, carotenoids, flavonoid, polyphenol content, and antioxidant activity of six different chilli pepper cultivars available in Nepal.

Material and Methods

Plant Materials

Six different chilli cultivars viz. *'Akabare'*, *'Jire'*, 'HRD-CHI-009', 'HRD-CHI-010', 'HRD-CHI-012', and 'HRD-CHI-014' were used in this study. Samples of 'HRD-CHI-009', 'HRD-CHI-010', 'HRD-CHI-012', and 'HRD-CHI-014'cultivars were obtained from Horticulture Research Division, Nepal Agricultural Research Council (NARC), whereas samples of *'Akabare'* and *'Jire'* cultivars were collected from the farmer's field of Dolakha and Lalitpur districts, respectively. Samples were taken at two different maturity stages: fully developed fruit just before the onset of turning color and at whole colored fully ripened stage.

Chemicals and Instrument

2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene standard (assay ≥93%) were purchased from Sigma-Aldrich Company, USA. Phenol reagent was purchased from Finar Limited, India. Gallic acid was purchased from LOBA Chemie India. Methanol and Petroleum ether were purchased from Fisher Scientific, India. Sodium carbonate, Aluminium trichloride and other required chemicals were purchased from Merck limited, India. All chemicals used were of analytical grade. The spectrophotometer used was of model GENESYSTM 10S Vis Spectrophotometer from Thermo Scientific TM, Germany.

Dry Matter Content

Dry matter content was calculated by determining moisture content from immiscible solvent distillation method according to the method described in Rangana (2011). 10 grams of fresh sample was boiled with 75ml toluene to carry out distillation.

$$\text{Dry matter \%} = 100 - \text{Moisture \%}$$

Oleoresin Content

Oleoresin content was analyzed by Soxhlet distillation method using Petroleum Ether according to Rangana (2011).

Polyphenol, Flavonoid, and Antioxidant Activity

Extract Preparation

The extracts of chilli pepper were prepared according to the method described by Dimitrijević et al. (2014) with some modification. 5g of sample was ground with 80 % methanol (30 mL), kept under continuous shaking for 20 minutes and filtered through Whatmann no. 1 filter paper. The residue was again submitted to two more extraction cycle for 20 minutes each totalizing 60 minutes of extraction time. The filtrate was combined in a volumetric flask, and the volume was made up to 100 ml with 80 % methanol. The extracts were stored in the refrigerator for analysis of polyphenol, flavonoids, and antioxidant activity.

Polyphenol Content

The total phenol content of sample extracts was measured by using Folin-Ciocalteu method, as described by Mahadavi et al. (2010). 1 ml of extract or standard solution of gallic acid (100 µg/ml to 1000 µg/ml) was decanted in 25 ml volumetric flask, which contained 9 ml of distilled water. 1 ml of Folin-Ciocalteu reagent was added to the mixture and shaken. After 5 minutes, 10 ml of 7% Na_2CO_3 solution was added and the solution was diluted to volume with distilled water and mixed well. After incubation for 90 min at room temperature, the absorbance against a prepared reagent blank (distilled water) was measured using a UV- VIS spectrophotometer at a wavelength of 765 nm. Standard solutions of gallic acid were used to obtain a standard curve. The results were expressed as mg of gallic acid equivalents (GAE) per 100g DW (dry weight) of the sample.

Flavonoid Content

The total flavonoid content (TFC) was determined as described by Samatha et al.(2012) and Walvekar and Kaimal (2014) using the aluminum chloride assay through spectrophotometry. An aliquot (0.5 ml) of extracts were taken in different test tubes and then 2 ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) and allowed to stand for 6 min. Later 0.15 ml of aluminum trichloride (10% $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) and final volume was made up to 5 ml with distilled water. After 15 min of incubation at room temperature, the mixture turns to pink, whose absorbance was measured using UV- VIS spectrophotometer at wavelength 510 nm. Distilled water was used as a blank. The calibration standard curve was prepared by preparing gallic acid solutions and results were expressed as mg of Gallic acid equivalents per 100g DW of the sample.

Antioxidant Activity

The antioxidant activity was determined by the DPPH radical scavenging method as described by Walvekar and Kaimal (2014) and Rajan et al.(2011). DPPH solution (0.004% w/v) was prepared in 95% methanol. The samples were mixed with 95% methanol in 1:9 ratios so as to make final volume 10 ml, thus the extract was prepared. An equal volume of extract and freshly prepared DPPH (0.004% w/v) were mixed and the tubes were incubated at room temperature in dark for 10 minutes and the absorbance was taken at wavelength 517 nm using a UV-Vis spectrophotometer. 95% methanol was used as a blank. The scavenging activity of the extract against the stable DPPH was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{A - B}{A} \times 100$$

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

Ascorbic Acid Content

The ascorbic acid content was determined by 2,6-dichlorophenol-indophenol visual titration method as described in Rangana (2011).

Total Carotenoid Content

The total carotenoid content of chilli pepper samples was determined as described by Rainha et al.(2011). Methanolic solutions of chilli pepper sample extracts were analyzed in UV-VIS spectrophotometer at wavelength 470, 653, and 666 nm. The concentrations of carotenoids and chlorophylls α and β were determined according to the equations reported by Lichtenthaler and Wellburn (1983) as follows:

$$\text{Total Carotenoids (mg/L)} = \frac{(1000 \times \text{Abs}470 - 2.860 \times C\alpha - 129.2 \times C\beta)}{2}$$

$$\text{Chlorophyll } \alpha \text{ (mg/L)} = 15.65 \times \text{Abs}666 - 7.340 \times \text{Abs}653$$

$$\text{Chlorophyll } \beta \text{ (mg/L)} = 27.05 \times \text{Abs}653 - 11.21 \times \text{Abs}666$$

Where Cα and Cβ are Chlorophyll α and Chlorophyll β.

β-carotene Content

The β-carotene content of the chilli pepper samples was determined by solvent partition method as described in Rangana (2011). Pigments (carotenoids and chlorophylls) in the sample were extracted in acetone and transferred to petroleum ether (xanthophylls have limited solubility in ether but are mostly soluble in alcohol). Chlorophylls are saponified with methanolic KOH, which is removed from the mixture by washing with distilled water. The amount of carotene in the ether extract is then determined by the spectrophotometric method at wavelength 450 nm against the β-carotene standard.

Statistical Analysis

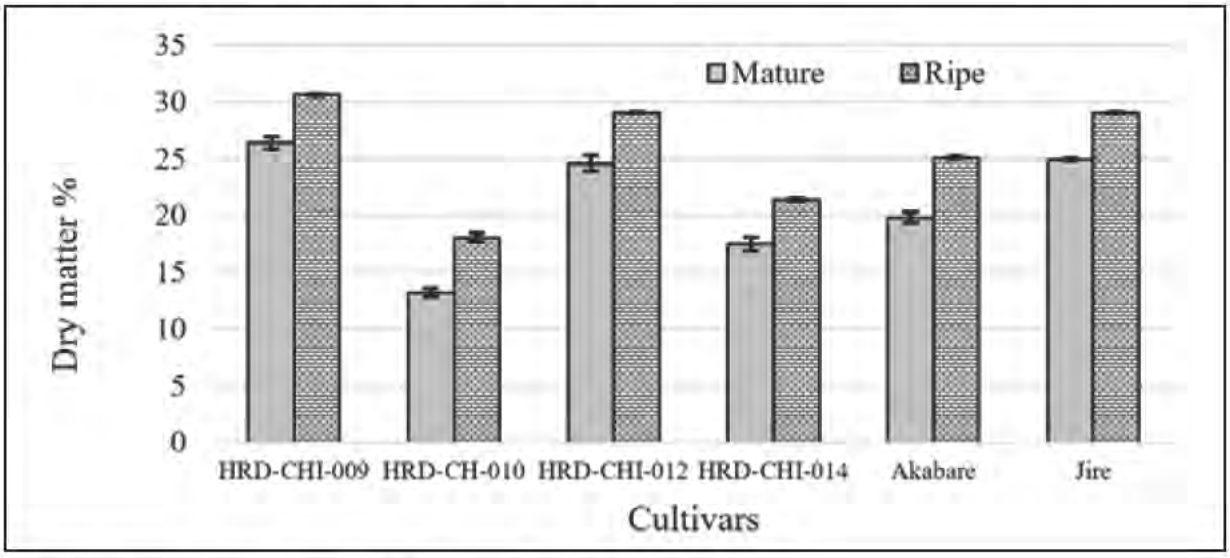
The data were analyzed by two factor analysis of variance (ANOVA) in a completely randomized design using statistical software package R Studio version 3.4.4. The mean and standard error of mean were calculated. Multiple comparisons of the treatment means were performed by LSD test at significance level of $P < 0.05$.

Results and Discussion

Dry Matter Content

There were variation among cultivars of chilli pepper for dry matter content (%) both at matured and ripened stage (Figure 1). The dry matter content followed an increasing trend from matured stage to ripened stage in all cultivars. Similar observation was reported by Niklis et al. (2002) during the study of change in dry matter content in sweet pepper fruit during different ripening stages. Khyadagi et al. (2012) and Nour et al. (2017) also reported continuous increase of moisture content of chilli with advancement in maturity stages. The increase of dry matter during advancement in maturity stages is related to ripening process of fruits, which was manifested through the synthesis of metabolites with complex molecular structure (Nour et al. 2017).

On overall comparison of average among cultivars at both maturity stages, dry matter content ranged from 15.62 to 28.49 (%) in HRD-CHI-10 and HRD-CHI-9 respectively.



*Values are the Mean ± Standard Error of Mean (SEM) obtained from triplicate data

Figure 1 Effect of cultivars and fruit maturity at harvest on dry matter content of chilli fruit

Oleoresin Content

There was a highly significant difference among chilli pepper cultivars for oleoresin content (%) both at matured and ripened stages, and a highly significant interaction between cultivars and maturity stages was also observed. The oleoresin content followed an increasing trend from matured stage to ripened stage in all cultivars as shown in

(Figure 2). Similar significant increase in oleoresin content in chilli with advancement of maturity was reported by Khyadagi et al. (2012). On overall comparison of average among cultivars at both maturity stages, oleoresin content ranged from 5.60 to 8.64 (%) in HRD-CHI-10 and *'Jire'* respectively. Similar observations of oleoresin content in the range of 2.26 to 13.76 (%) in Mexican chilli peppers were reported by Sepulveda et al. (2013).

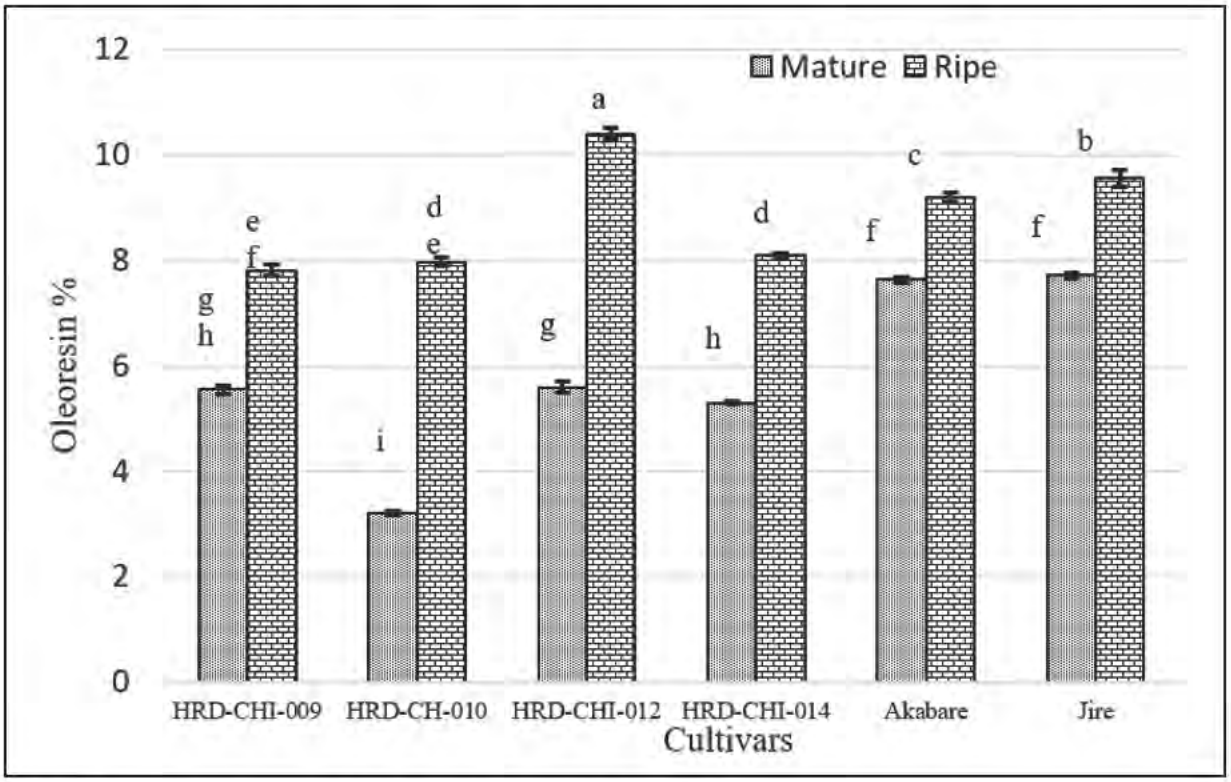


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Figure 2 Effect of cultivar and fruit maturity at harvest on oleoresin content of chilli fruit

Ascorbic Acid Content

Ascorbic acid content increased with the advancement in maturity from matured stage to ripened stage in all cultivars (Figure 3). These results were similar to data reported by Osuna-Gracia et al. (1998); Martinez et al.; (2005); and Kumar and Tata (2009). In all cultivars ascorbic acid was positively correlated with dry matter content which shows a strong relationship in the maturation and ripening related changes in ascorbic acid. Considering that glucose is the precursor in ascorbic acid synthesis (Davely et al. 2000; Niklis et al. 2002) the positive correlation is justifiable (Nour et al.2017). Similarly, ascorbic acid content at range of 72 to 208 mg/100gm (green stage) and 137.50 to 280 mg/100gm (red stage) on eighteen different chilli pepper genotypes was reported by Tata et al. (2009). Howard et al. (1994) also reported up to 95% increase of ascorbic acid content in red peppers than in green stage. Gnayfeedet al. (2001) and Ghasemnezhad et al. (2011) have reported increase of ascorbic acid content at color break stage and declined with further ripening. This could be due to the antioxidant role of ascorbic acid which increases with increasing respiration rate of the fruit during ripening stage (Markuset al.1999). On overall comparison of average at both maturity stages, cultivars showed significant variation in ascorbic acid content which ranged from 76.36 to 209.22 mg/100g in HRD-CHI-014 and HRD-CHI-012 cultivars, respectively. A similar observation on ascorbic acid content at range of 72 to 208 mg/100gm (green stage) and 137.50 to 280 mg/100gm (red stage) on eighteen different chili pepper genotypes was reported by Tata et al. (2009).

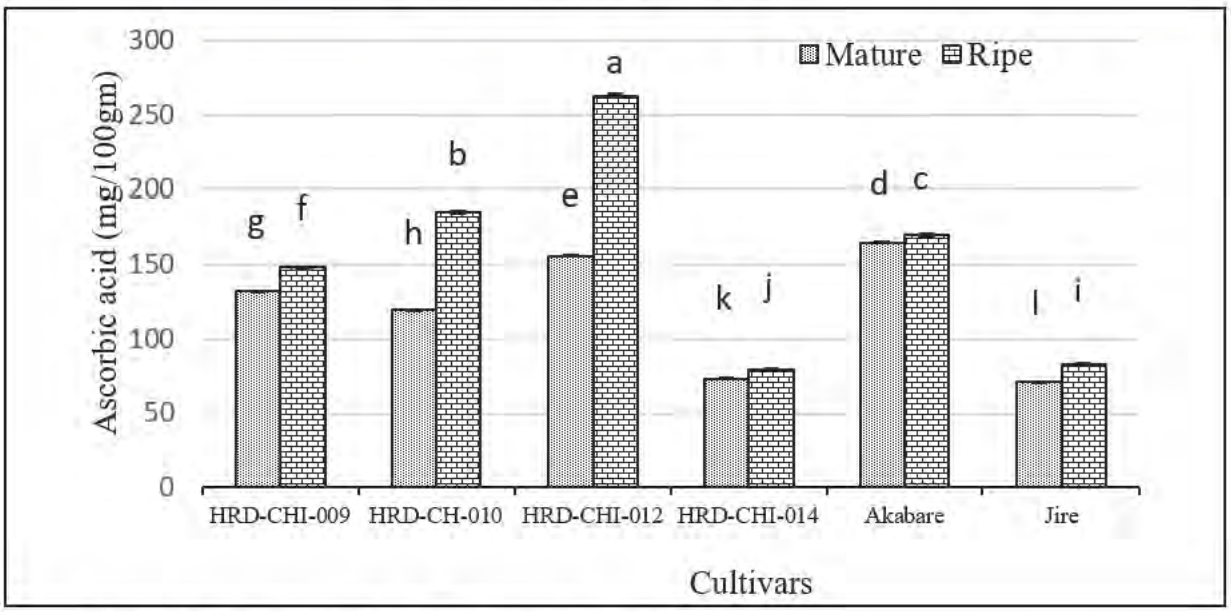

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Figure 3 Effect of cultivar and fruit maturity at harvest on ascorbic acid content of chilli fruit

Flavonoids Content

There was a highly significant difference among chilli pepper cultivars for flavonoids content both at matured stage and ripened stages and a highly significant interaction between cultivars and maturity stages was also observed. As shown in figure 4, the flavonoids content followed a decreasing trend from matured stage to ripened stage. From overall comparison of average among cultivars at both maturity stages, flavonoids content ranged from 80.71 to 149.43 (GAE mg/100gm) in HRD-CHI-12 and *'Akabare'* cultivars respectively. The changes in flavonoids content were dependent on pepper cultivars (Ghasemnezhad et al. 2011). Sukrasno and Yeoman (1993) and Howard et al. (2000) also reported higher flavonoids content in green chilli peppers which decreased as the chilli pepper ripened. A high concentration of flavonoid (quercetin) in green pepper fruits is associated with functions of protecting photosynthesis mechanism as it strongly absorbs radiations within the range of 280-315 nm acting as filters of UV radiation (Harborne and Williams, 2000). When chlorophylls are disintegrated photosynthesis process stops leading to decrease of flavonoid (quercetin) in ripe pepper (Materska and Peruka, 2004).

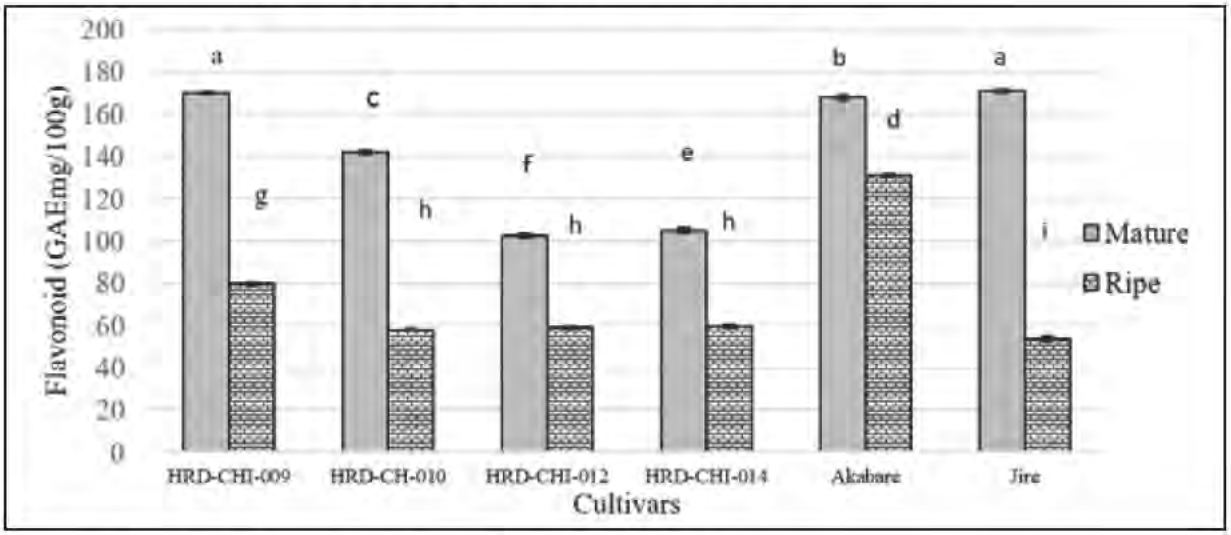

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Figure 4 Effect of cultivar and fruit maturity at harvest onflavonoid content of chilli fruit

Antioxidant Activity

Antioxidant activity is an important parameter to establish the health functionality of a food product (Kaur and Kapoor, 2001). Significant difference among cultivars, maturity stages and significant interaction among cultivars and maturity stages was observed (Fig. 5). From overall comparison of average among cultivars and maturity stages, antioxidant activity ranged from 21.20 to 64.18 (% DPPH scavenging) in HRD-CHI-10 and *'Akabare'* cultivars respectively. Antioxidant activity increased significantly from matured stage to ripened stage on all cultivars. Similar increase in antioxidant activity of pepper cultivars with advance in maturity stages were reported by Howard et al. (2000), Navarro et al. (2006), Ghasemnezhad et al. (2011) and Nour et al. (2017). Enhanced antioxidant activity at fully ripened stage reflects the importance of consuming pepper fruits at this stage.

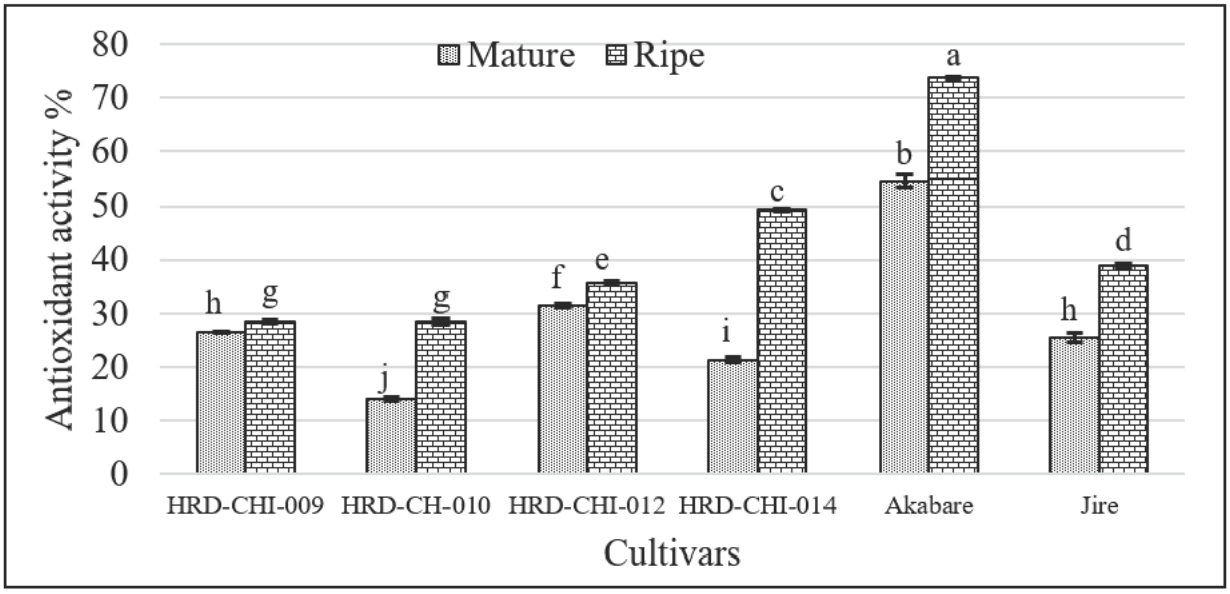

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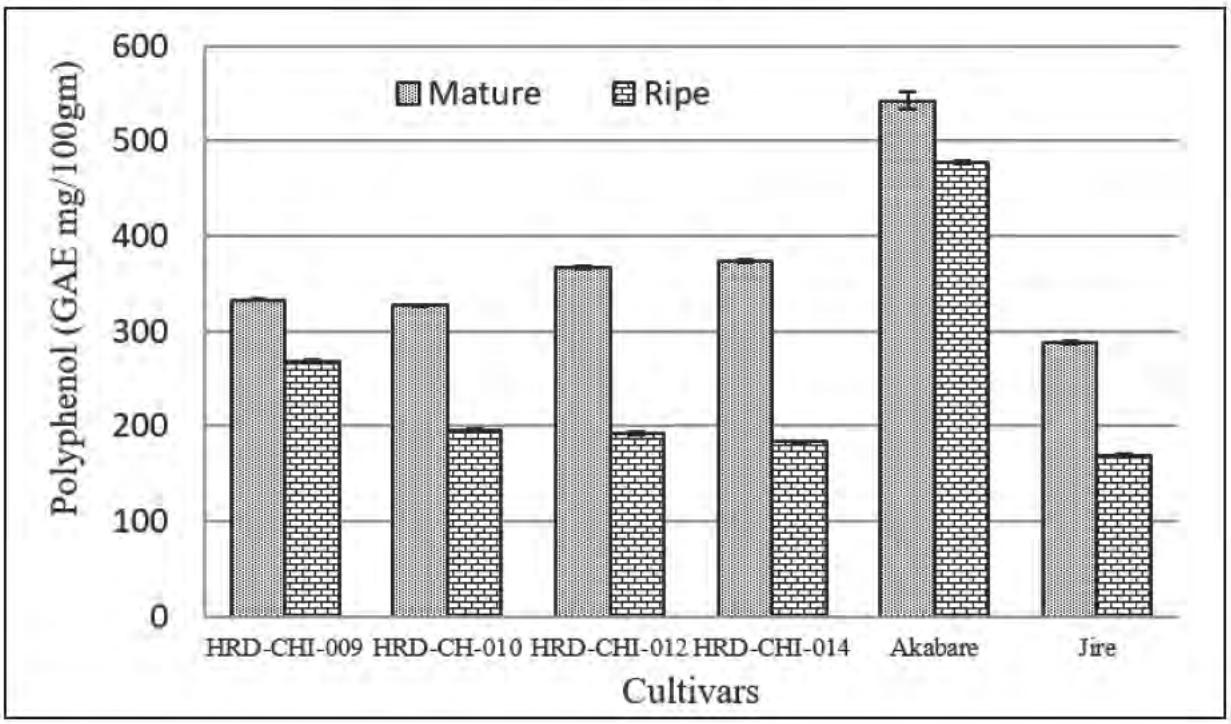
Figure 5 Effect of cultivar and fruit maturity at harvest on antioxidant activity of chilli fruit (shown by 10mg/ml extract)

Polyphenol Content

In this study polyphenol content was influenced by cultivars and maturity (Figure 6). On overall comparison of average among cultivars and maturity stages, polyphenol content ranged from 228.07 to 510.46 (GAE mg/100gm) in *'Jire'* and *'Akabare'* cultivars respectively. Fruits of all pepper cultivars harvested at matured stage had more polyphenol content in comparison to pepper fruits harvested at ripened stage. Although an increase of polyphenol content in pepper fruits during maturation has been reported by Leet al. (1995) however, when ascorbic acid concentration is high the polyphenol content could be overestimated due to the response of ascorbic acid to the Folin-Ciocalteau assay (Navarro et al. 2006). In the present study, fully ripened fruits had less polyphenol content than fully developed and matured fruits. This result is in agreement with findings made by Conforti et al. (2007) and Ghasemnezhad et al. (2011), wherein decrease of phenolic contents with increase in maturity and following ripening were reported. Howard et al. (2000) have reported that change in phenolic content depends on pepper cultivar which is also observed in this study. Losses of polyphenol content during fully ripened stage of some pepper cultivars were related to activity of peroxidase (Gnayfeed et al.2001).



*Values are the Mean ± Standard Error of Mean (SEM) obtained from triplicate data

Figure 6 Effect of cultivar and fruit maturity at harvest on polyphenol content of chilli fruit

Beta Carotene and Total Carotenoids

There was a highly significant difference among chilli pepper cultivars for total carotenoids content both at matured stage and ripened stage and a highly significant interaction between cultivars and maturity stages was also observed. Beta carotene content did not differ significantly among the cultivars at matured stage but were significantly different at ripened stage. The beta carotene and total carotenoids content followed an increasing trend from matured stage to ripened stage in all cultivars (Figure 7 and 8). From comparison of average among cultivars at both maturity stages, beta carotene content ranged from 0.20 to 0.99 (mg/100gm) in HRD-CHI-10 and *'Akabare'* cultivars respectively, which were supported by the findings made by Howard et al (1994). Similarly, total carotenoids content ranged from 2459.95 to 4543.86 (mg/100gm) in HRD-CHI-14 and *'Akabare'* cultivars respectively. Similar significant increase in beta carotene and total carotenoids content in chilli with advancement of maturity was reported by Gnayfeedet al. (2001) and Markus et al. (1999). On observation made by Gnayfeed et al. (2001) beta carotene and total carotenoids content were reported in the range of 2.1 to 3.6 (mg/100gm) and 752.1 to 1218.3 (mg/100gm) respectively. In addition, Markus et al. (1999) reported total carotenoids content in pepper fruit at range of 677 mg/100gm (green stage) to 1210.80 mg/100gm (deep red stage), which supported our finding of increase in total carotenoids with advancement of maturity stages. Increase in beta carotene concentration is the result of light-independent carotenogenesis and role of stability of carotenoids in red pepper depends to a considerable amount to the genotypes (Gnayfeed et al. 2001).

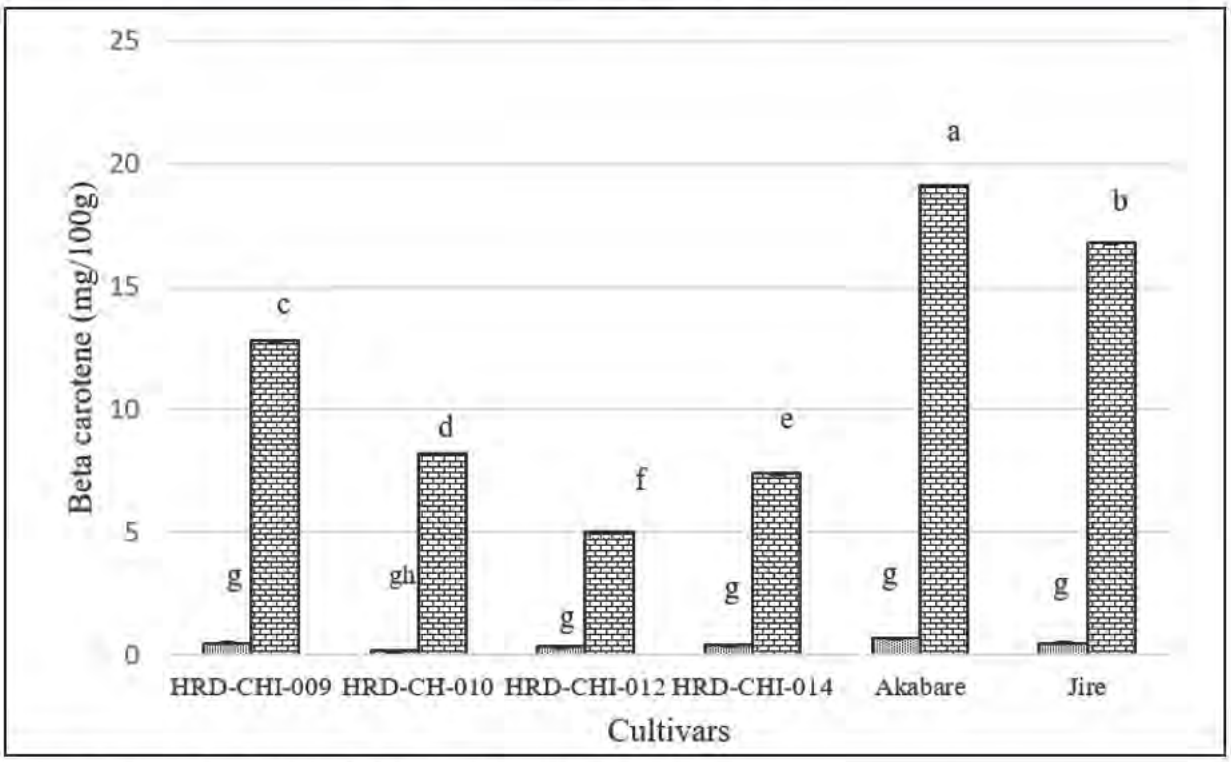


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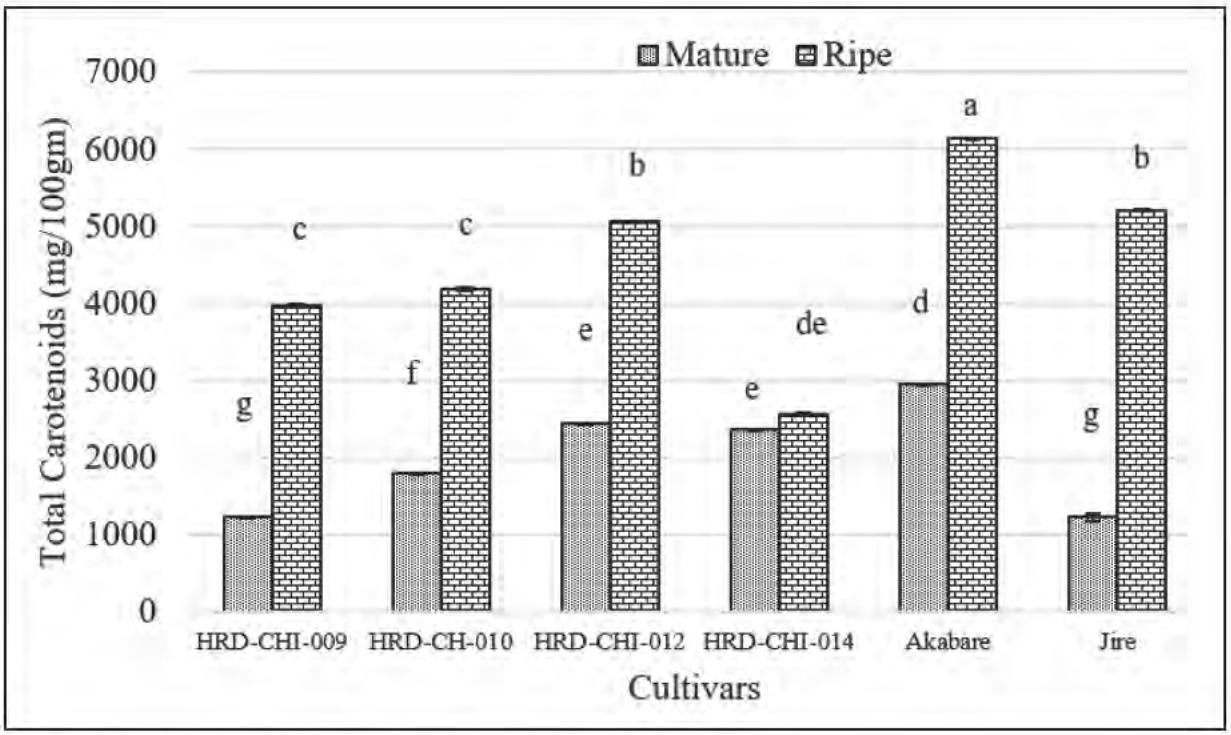
Figure 7 Effect of cultivar and fruit maturity at harvest on beta carotene content of chilli fruit



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**Values with different superscripts in a column differs ($p<0.001$)

Figure 8 Effect of cultivar and fruit maturity at harvest on total carotenoids content of chilli fruit

Conclusion

From this study, wide variation in dry matter, oleoresin, ascorbic acid, flavonoid, polyphenol, antioxidant activity, beta carotene and total carotenoid content among different chilli pepper cultivars was determined. These bioactive compounds were greatly influenced by maturity stages. With advanced maturation dry matter, oleoresin, ascorbic acid, beta carotene, total carotenoids, and antioxidant activity increased; however, polyphenol and flavonoid contents decreased. Among the six different chilli cultivars used in this study, *Akabare* cultivar was found to be the best cultivar from the aspect of flavonoid, polyphenol, beta carotene, and total carotenoid contents. Similarly, HRD-CHI-012 and HRD-CHI-009 were the best cultivars for ascorbic acid and dry matter contents, respectively. Consumption of chilli pepper at ripened (red colored) stage was found to be more appropriate as it contained higher amount of health promoting bioactive compounds than in green stage.

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