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Research Article

Evaluation of Biochemical Traits of Three Sweet Pepper (*Capsicum annuum* L.) Varieties in Bangladesh

Arifa Setu, Farjana Akter, Md. Mahadi Hasan, Sudipto Datta, Shayla Sharmin, and Muhammad Javidul Haque Bhuiyan✉

Department of Biochemistry and Molecular Biology, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

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Correspondence
Muhammad Javidul Haque Bhuiyan
✉: mjhbhuiyan@bau.edu.bd

ABSTRACT

Three hybrid varieties (F_1; green, yellow and red colour) of sweet pepper (*Capsicum annum*), which possess great economical and nutritional potentialities, were taken in this study to assess their nutritional quality based on some biochemical parameters. Fresh capsicum of all three varieties were directly purchased from the greenhouse-shed of Shaurav Fishers and Agro Ltd, Trishal, Mymensingh. Vitamin C was determined by redox titrate method, total sugar (620 nm, anthrone solution), reducing sugar (510nm, dinitro salicylic acid solution), β-carotene (510nm, 480nm, acetone solution) and lycopene (663nm, 645nm, 505nm, 453nm, acetone and hexane solution) were estimated using a UV-visible spectrophotometer in the post-graduate laboratory of Bangladesh Agricultural university, Mymensingh. All the varieties showed variations significantly ($p \leq 0.05$) among the studied parameters set with complete randomized design. The range of mean performance for the following biochemical parameters was determined to be: vitamin C 136.151–252.89 mg, titratable acidity 0.1–0.6%, total sugar 0.99–2.23%, reducing sugar 1.51- 2.35%, βeta–carotene 1.03-8.40 μg g^{-1} and lycopene 0.001–0.15 mg 100 ml^{-1}. According to Duncan Multiple Range Test the parameters showed efficiency at 1% level of significance. From the overall ranking based on efficiency score red capsicum was the best one followed by yellow and green. Therefore, it could be concluded that among the three-capsicum varieties, the red variety possess more nutritional value.

Introduction

Capsicum, also known as bell pepper or sweet pepper, belongs to the Solanaceae family(Howard et al., 2000). It comes from the South and Central America. Approximately thirty wild varieties and five cultivated species, including *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens*, *Capsicum annuum*, and *Capsicum pubescens*, are found in the genus Capsicum (DeWitt & Bosland, 1996). The fleshy, hollow fruits of capsicum species are recognizable by their diverse shapes, sizes, and hues, which range from green to red, yellow, orange, and even purple. Capsicum, widely regarded for its vibrant colours and crisp texture, and essential nutrients. It is particularly rich in vitamin C, with red capsicum varieties containing up to three times more vitamin C than oranges, making them an excellent source of antioxidants that support immune health and skin vitality (González-Zamora et al., 2013). Additionally, capsicum is a significant source of vitamin A, primarily in the form of β-carotene, which is essential for maintaining healthy vision and promoting skin health. The different colours of capsicum - green, yellow, and red reflect varying levels of nutrients; for instance, red capsicums contain higher concentrations of lycopene, a potent antioxidant linked to reduced risks of certain cancers (Johnson, 2002). Beyond these vitamins, it provides a moderate amount of dietary fibre, aiding in digestion and contributing to heart health. It is also practically fat-free and low in calories, which makes it a good addition to a balanced diet, especially for people trying to control their weight or increase the amount of nutrient-dense foods in their meals (Howard et al., 2000). It also contains a trace amount of omega-3s and omega-6s fatty acids. Each 100 g of edible capsicum flesh has 24 kcal of energy and 1.3 g, 4.3 g, and 0.3 g of protein, carbohydrate, and fat, respectively (Haque et al., 2019).

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In Bangladesh, capsicum was just recently introduced, mostly as a result of agricultural development initiatives meant to diversify crop production (Islam et al., 2021). In the early 2000s, capsicum was a very tiny crop when it was first grown, producing barely 500 metric tons annually. With support from non-governmental organizations and government action through the Department of Agricultural Extension (DAE), things started to change in the middle of the 2010s. These groups trained farmers, introduced high-yield cultivars, and improved pest management strategies (Islam et al., 2021). As a result, Bangladesh's capsicum production rose substantially, reaching over 3,000 metric tons by 2015. The world's largest manufacturer is China, making up about 33% of the entire production area and nearly 50% of global production.

In recognition of its many culinary uses, capsicum has become increasingly popular among Bangladeshi consumers. From classic curries to contemporary salads, the vegetable is utilized in a variety of recipes, and its bright colours and healthful qualities have made it a popular component. Market prices, which have been advantageous for farmers, have mirrored this rising demand. Compared to many other vegetables that are often grown in the nation, capsicum typically commands a farm gate price of BDT 60 to BDT 80 kg^{-1}. Because of its price stability and a very short growing season, which permits several harvests in a single year, capsicum is a desirable crop for growers trying to optimize their profits. With the various potentials of capsicum in mind, the current study was conducted to evaluate the biochemical properties (Vitamin C, titratable acidity, total sugar, reducing sugar, b-carotene, and lycopene) in order to compare the biochemical contents of the three coloured capsicum varieties and identify which was nutritionally rich.

Materials and Methods

Location, time, collection and sampling procedure

The freshly harvested capsicum cv. Green, Red, Yellow–F_1 hybrid were collected from Shaurav Fisheries & Agro Ltd, Trishal, Mymensingh. A total of 50 mature, uniform sized, undamaged healthy fruits were selected for the study. The investigation was conducted in the Professor Mohammad Hossain laboratory of the Department of Biochemistry and Molecular Biology during October 2022 to March 2023. Variations of biochemical traits such as Vitamin–C, titratable acidity, total sugar, reducing sugar, beta (β)–carotene and lycopene content among different coloured hybrid F_1 lines were performed to assess their quality.

Estimation of Vitamin–C

The vitamin-C content of capsicum was estimated by the method of AOAC (2016). Five grams of fresh capsicum pulp and 100 ml of 6% metaphosphoric acid solution were blended well in a blender. It was filtered after blending and put into the 100 ml beaker. Five millilitres of the sample were placed in a conical flask and titrated using dye solution. The following formula was used to determine the samples' vitamin C content:

$$\text{Vitamin-C}\left(\frac{mg}{100g}\right) = \left(\frac{\textit{Titre} \times \textit{dye factor} \times \textit{made volume up.}}{\textit{Aliquot of extract taken for estimation} \times \textit{wt of a sample volume}}\right)$$

Titratable Acidity

Titratable acidity was measured by the method of (AOAC, 2000). Ten grams of Capsicum were blended with 200 ml of distilled water in a blender (Family mate, Japan, India). After thoroughly blending the sample, it was moved into a 500 ml beaker and heated on a hot plate for 40 minutes. Switching off the hot plate, the beaker on the hot plate was kept for 20 minutes and made volume up to 250 ml.5 ml of the sample was put into a conical flask and a micro burette was filled with 0.1N NaOH solution. Then the mixed solution was titrated with dye using a phenolphthalein indicator solution to a pink coloured endpoint. The titratable acidity content of the samples was calculated by using the following formula:

$$\text{Titratable Acidity} = \left(\frac{\textit{Titrate} \times \textit{normality of alkali} \times 1000 \times \textit{equivalent weight of acid}}{\textit{Sample weight}}\right)$$

Estimation of total sugar

The total sugar content of Capsicum pulp was determined spectrophotometrically by the anthrone method as described by Jayaraman and Jayaraman (1981). Sugar was extracted from capsicum pulp using the following technique (Loomis & Shull, 1937). One g of pulp was taken and homogenized with ethyl alcohol and boiled for 10 minutes 70% of 10 ml ethyl alcohol. About one-fourth of ethyl alcohol was evaporated and cooled. Then it was filtered with cloth and the remaining aqueous sample solution was carefully put into a 100 ml volumetric flask and made volume up to the mark. Then 1 ml of sample solution was transferred from the volumetric flask to a test tube and add 9 ml distilled water to make a 10 ml sample solution. An aliquot of 1 ml of sample solution was taken in a test tube, and 4 ml of anthrone reagent was added and mixed thoroughly. Then test tubes were placed in a boiling water bath and boiled for 5 minutes. Four millilitres of anthrone reagent and one millilitre of water were combined in a test tube to create a blank reagent, which was then handled in the same way. A spectrophotometer ((T80, PG Instruments, UK) was used to measure the blue-green solution's absorbance at 620 nm. The absorbance was done in triplicate. The test tubes containing 0.0, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 mg of glucose each were filled with 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml of standard glucose solution. The volume was then increased to 1 ml with distilled water to create a standard glucose curve). Each test tube was then filled with 4 ml of anthrone reagent, which was thoroughly mixed. As previously said, each of these options was handled similarly. At 620 nm, the absorbance was measured using a blank that contained 4 ml of anthrone reagent and 1 ml of water. The amount of total sugar was calculated from the standard curve of glucose. The percent total sugar was calculated as follows:

$$\text{Total sugar (\%)} = \left(\frac{Amount\ of\ sugar\ obtained}{Weight\ of\ pulp}\right) \times 100$$

Determination of reducing sugar

Reducing the sugar of Capsicum was determined by the Dinitro alicyclic acid method (Miller, 1959). One g of pulp was taken and homogenized with ethyl alcohol and boiled for 10 minutes 70% of 10 ml ethyl alcohol. About one-fourth of ethyl alcohol was evaporated and cooled. Then it was filtered with markin cloth, and the remaining aqueous sample solution was carefully put into a 100 ml volumetric flask and made the volume up to the mark. Then, to create a 10 ml sample solution, 1 ml of the sample solution was transferred from the volumetric flask into the test tube and 9 ml of distilled water was added. Three millilitres of extract were pipetted into a test tube, and each of these solutions was mixed with three millilitres of DNS reagent for five seconds using a vortex machine. At medium heat, the test tubes were cooked for five minutes. When the colour appeared and the contents of the test tubes were still warm, 1 millilitre of 40% Rochelle Salt was added. Water from the faucet was used to chill the test tubes. Three millilitres of distilled water and three millilitres of DNS reagent were combined in a tube to create a reagent blank. A colorimeter was used to measure the solution's absorbance at 540 nm. The glucose standard curve was used to determine the amount of reducing sugar. The percentage of reducing sugar present in the Capsicum was determined by using the following formula:

Percentage of reducing sugar (g 100–1 g) =

$$\left(\frac{\%\ reducing\ sugar\ \left(\frac{g}{100g}\right)}{Weight\ of\ pulp}\right) \times 100$$

Estimation of Beta (β)–Carotene

Beta-carotene of Capsicum was determined by the method of Sarker and Oba (2020). A mortar and pestle was used to thoroughly grind five grams of fresh capsicum sample with ten millilitres of 80% acetone. The extract was centrifuged at 10,000 × g for three to four minutes after the supernatant was removed and placed in a falcon tube. The volume was raised to 20 millilitres. At 510 and 480 nm, the absorbance was measured with a spectrophotometer. Beta-carotene levels were reported as $\mu g g^{-1}$ fresh weight. The following formula was used to estimate the amount of β-carotene.

$$\beta\text{– carotene} = \frac{7.6\ (Abs.at\ 480\ nm) - 1.49\ (Abs.at\ 510\ nm) \times\ final\ volume}{1000 \times\ fresh\ weight\ of\ Capsicum}$$

Estimation of lycopene

After extracting one gram of fresh capsicum fruit twice in ten millilitres of a 4:6 acetone: n-hexane combination, the mixture was allowed to stand in an ice bath for ten minutes. The Hettich EBA 21 centrifuge was then used for 10 minutes at 1370× g. An UV–vis spectrophotometer (T80, PG Instruments, UK) was used to spectrophotometrically measure the absorbance of the hydrophobic fraction at wavelengths of 663,645,505, and 453 nm. Each fruit was measured in three replicates. The following equation, which was put forth by Nagata and Yamashita (1992), was used to measure the lycopene concentration:

$$\text{Lycopene (mg 100 ml– 1)} = -\ 0.0458\ A663\ +\ 0.204\ A645\ +\ 0.372\ A5050 -\ 0.0$$

Statistical analysis

Statistical analyses were performed with the statistical tool STAR (version 2.0.1). The F-test was used to perform analysis of variance (ANOVA) for all parameters after the means were determined for each treatment (*capsicum* spp.). DMRT (Duncan's Multiple Range Test) was used to compare the means of various parameters. The least significant difference (LSD) test was used to examine the significance of the difference between the mean pairings at both the 1% and 5% probability levels.

Results

Vitamin C

A significant variation of vitamin C content was observed among the three varieties in Figure 1. There was no significant variation between green capsicum and yellow capsicum, however, each of them showed significant variation (at 5% level) from red capsicum. Upon comprehensive evaluation of the three varieties, it is clear that red capsicum exhibited the highest mean vitamin C content (252.89 mg%). followed by green capsicum (173.58 mg%) and yellow capsicum (136.15mg %).

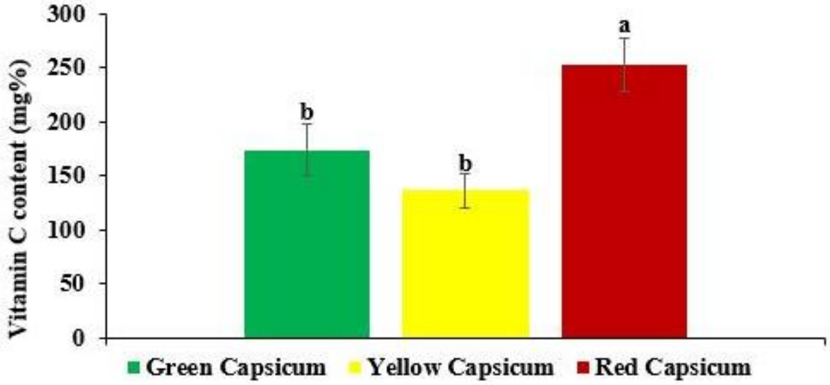

Figure 1. Comparison of Vitamin C content (mg%) on three selected capsicum varieties. The vertical bars represent the mean ± SEM values of capsicum varieties. Data are labelled with standard error bars along with the LSD 5% level of significance. Bars showing (a–b) letters are significantly different between varieties at $p \leq 0.01$ (ANOVA, DMRT Test).

Titratable Acidity

A statistically significant variation (at 5% level of significant) of titratable acidity percentage was observed among three selected capsicum varieties in Figure 2. Green capsicum showed significant variation to both the yellow capsicum and red capsicum, while yellow capsicum showed no significant difference with the red capsicum. It is further observed that green capsicum exhibited the highest titratable acidity value (0.61%). On the other hand, yellow capsicum had the lowest value (0.14%) and red capsicum showed intermediate values (0.21%) according to Figure 2.

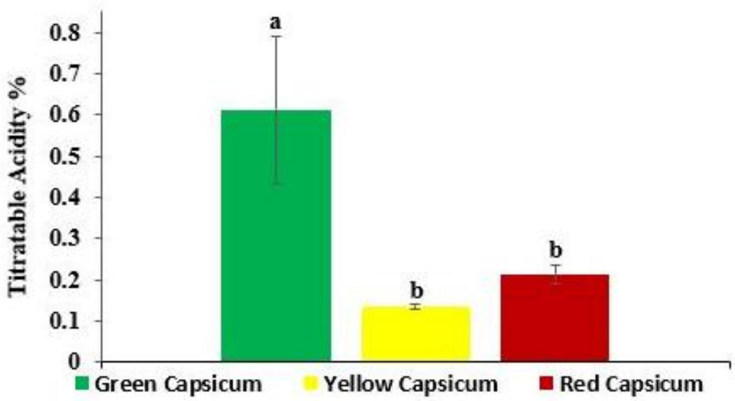

Figure 2. Comparison of Titratable acidity percentage on three selected capsicum varieties. The vertical bars represent the mean ± SEM values of capsicum varieties. Data are labelled with standard error bars along with the LSD 5% level of significance. Bars showing (a–b) letters are significantly different between varieties at $p \leq 0.01$ (ANOVA, DMRT Test).

Total sugar

Total sugar content of the selected capsicum varieties was observed as significantly different in Figure 3. Green capsicum showed similar response as like red capsicum, but yellow capsicum showed a significant variation from both the green capsicum and red capsicum. According to Figure 3, it is clear that green and red capsicum exhibited almost similar total sugar content of 1.96% and 2.23%, respectively. On the contrary, yellow capsicum exhibited the lowest total sugar content (0.99%).

Reducing sugar

The study revealed significant (5% level) variation in reducing sugar content among the three selected varieties, as depicted in Figure 4. Red capsicum showed significant difference from both the green capsicum and yellow capsicum. However, there was no significant difference between green capsicum and red capsicum. Upon meticulous evaluation, it is found that red capsicum exhibited the highest reducing sugar content (2.35%) compared to both the green capsicum (1.51%) and the yellow capsicum (1.55%).

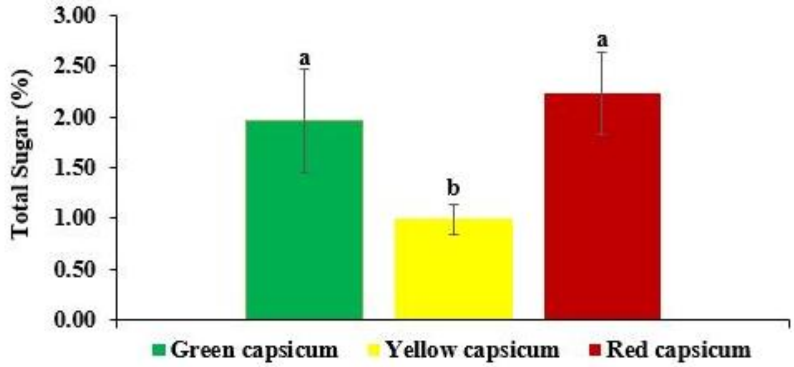

Figure 3. Comparison of Total sugar content on three selected capsicum varieties. The vertical bars represent the mean ± SEM values of capsicum varieties. Data are labelled with standard error bars along with the LSD 5% level of significance. Bars showing (a–b) letters are significantly different between varieties at $p \leq 0.01$ (ANOVA, DMRT Test).

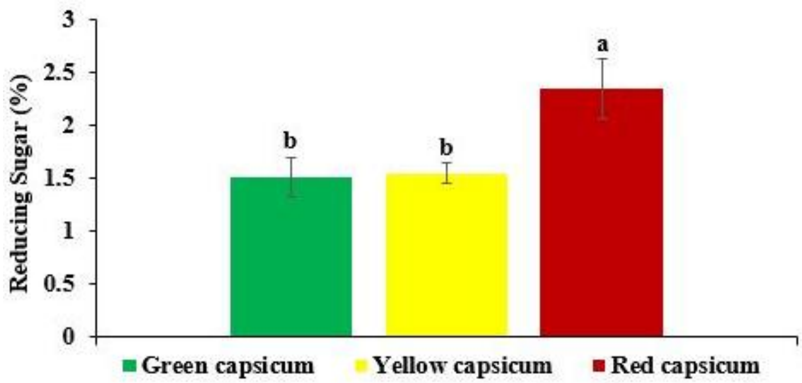

Figure 4. Comparison of Reducing sugar content on three selected capsicum varieties. The vertical bars represent the mean ± SEM values of capsicum varieties. Data are labelled with standard error bars along with the LSD 5% level of significance. Bars showing (a–b) letters are significantly different between varieties at $p \leq 0.01$ (ANOVA, DMRT Test).

Beta Carotene

Results of the paired mean comparison test (by DMRT) on the βeta–carotene content are shown in Figure 5. Ranking a, b, and c correspond to the significantly distinct variation. Red capsicum had the highest beta carotene content (8.40 μg g^{-1}) compared to yellow capsicum (4.29 μg g^{-1}) and further followed by green capsicum (1.033 μg g^{-1}).

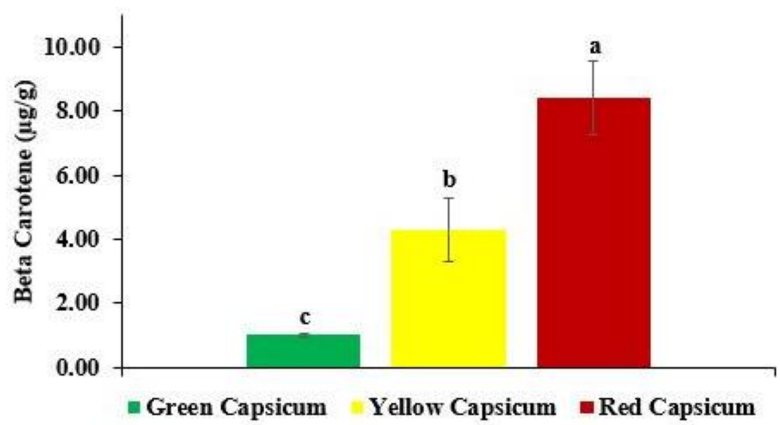

Figure 5. Comparison of β Carotene content percentage on three selected capsicum varieties. The vertical bars represent the mean ± SEM values of capsicum varieties. Data are labelled with standard error bars along with the LSD 5% level of significance. Bars showing (a–c) letters are significantly different between varieties at $p \leq 0.01$ (ANOVA, DMRT Test).

Lycopene

The study on the lycopene content revealed a significant variation (5% level of significance) among three selected varieties as depicted in Figure 6. The letters a and b indicate the significant distinct variations. Red capsicum exhibited the highest lycopene content (0.15 mg ml^{-1}) which was significantly varied from yellow capsicum (0.007 mg ml^{-1}) and green capsicum (0.001 mg ml^{-1}).

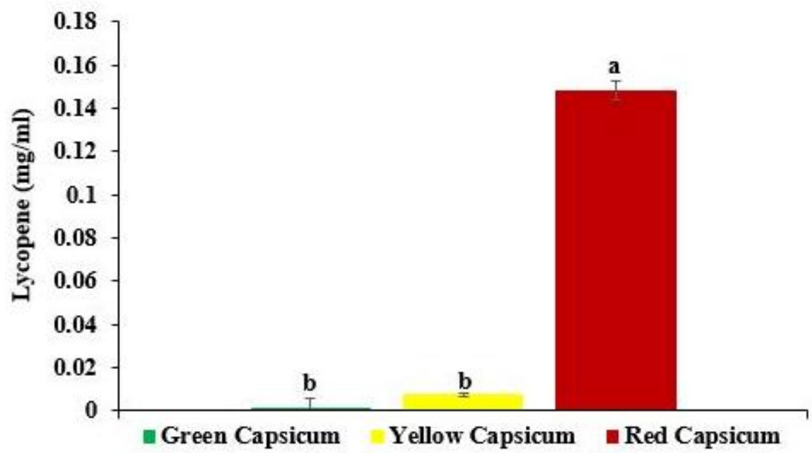

Figure 6. Comparison of Lycopene content percentage on three selected capsicum varieties. The vertical bars represent the mean ± SEM values of capsicum varieties. Data are labelled with standard error bars along with the LSD 5% level of significance. Bars showing (a–b) letters are significantly different between varieties at $p \leq 0.01$ (ANOVA, DMRT Test).

Discussion

Being a vital nutrient with antioxidant properties, vitamin C has an important role in developing the immune system, collagen synthesis, exhibiting nutritional diversity, and maintaining proper human health. In the present investigation, significant variation (5% level of significance) was observed in vitamin C content among all varieties. According to DMRT, the red capsicum had the highest level of vitamin C, which was statistically significant (1% level) from green and yellow varieties. Previous studies (Igbokwe *et al*., 2013; Ozgur *et al*., 2011) showed that vitamin C content was significantly the highest (ranged from 204 mg/100g to 280 mg/100 g) in red capsicum compared to green and yellow capsicum. Several scientists found that vitamin C content was exhibited in different levels due to varieties, growth conditions (temperature, soil, fertilizer), harvesting, post-harvest (storage and processing), and maturity stages (Lee & Kader, 2000). Therefore, in this research work, three hybrid F1 varieties of green, yellow, and red capsicum could be the reason for vitamin C variation.

The characteristic flavour of each capsicum variety is associated as a with the presence of titratable acid, primarily organic acids such as citrate and malate (Kidmose *et al*., 2006). In this study, the titratable acidity showed lower values in yellow and red varieties and higher values in green varieties. A previous investigation (Rodica *et al*., 2017) reported that the bell pepper, with green and yellow fruit, showed lower values of 0.448% (Figaro F1 and Cecil F1) and higher values of 0.704% in sweet peppers with red fruits. Ozgur et al. (2011) investigated that the titratable acidity percentage of capsicum depends on the type of variety, fresh or dried sample, environment, soil type, and stage of maturity. A moderate acidity can guarantee appropriate flavour, fragrance, etc., for a time. Thus, the acidity level might be used to rank the best variety from this trial.

Total sugar and reducing sugar are considered quality parameters due to their impact on sweet taste, colour, and texture modification. Total sugar includes reducing sugar (e.g., glucose and fructose) and non-reducing sugar (mostly polysaccharides). Higher total sugar and reducing sugar indicate the best quality of fruit. Considering total sugar, the green and red capsicum had the highest level of total sugar content, which was significantly different from the yellow variety. On the other hand, in the case of reducing sugar, red capsicum expressed itself as the best performer with high content of reducing sugar compared to green and yellow varieties. Different genetic factors contribute to variations in sugar content. Similar trends were also studied by Singh *et al*. (2013). Some studies reported a combined effect of the genotype and location in the nutritional composition of *Capsicum* peppers (Speranza *et al*., 2019), which can be remarkable in genotypes selected for specific local conditions, such as landraces.

Capsicum species synthesize and accumulate several carotenoid pigments such as β–carotene, α–carotene, γ–carotene, and β–cryptoxanthin, which are responsible for the fruits' yellow, orange, and red colours. Beta carotene acts as a precursor for provitamin A, which protects eyesight, reduces the risk of cancer, and suppresses tumorigenesis in the skin, lung, liver, and colon (Nishino *et al*., 2009). In this study, red capsicum exhibited the highest level of beta carotene content, which was statistically significant from the other varieties. Several studies evaluated by Fratianni *et al*. (2020) align with this result. In this experiment, beta–carotene variation would be observed due to varietal variations.

Lycopene, a powerful antioxidant that might help protect cells from damage, improve heart health, prevent sunburn, and protect against certain cancers, possibly prostate cancer (Johnson, 2002). It has been linked to the red colour of fruits. The higher lycopene content was found in red capsicum, which matched with the result documented in previous research (Ozgur *et al*., 2011), whereas lycopene content was close to zero in green and red varieties due to genetic mutation that affects the biosynthesis of lycopene.

The variety itself represents a genetic make-up. The performance was based on the genetic make-up of the heredity. Considering their genetic make-up, individual varieties had distinct biochemical characteristics. For this reason, results varied among the varieties. These variations accounted for varietal differences. When a variety is released, these parameters are genetically set within it. So, if there is no influence from any other treatment, it continues to give the values in the same manner. The link between genetic make-up and nutrient content is well established, and the variation observed in this study could be attributed to the genetic diversity among the varieties. According to Variety, the data on biochemical parameters varies due to differences in their genetic make-up. As they were genetically different, their expressions were different.

Conclusion

The investigation was conducted to analyse the biochemical parameters of three capsicum varieties, such as Green, Yellow, and Red, as the determinants of the variation in nutrient composition. The results of this investigation showed differences in the content of biochemical compounds in three capsicum varieties. The red capsicum had higher contents of vitamin C, total sugar, reducing sugar, beta carotene, and lycopene, except titratable acidity, and showed greater antioxidant capacity than did other varieties. The presence of an intermediate titratable acidity in red capsicum made it the best performer from the others due to maintaining the p^H value. There has been an inverse relationship between titratable acidity and p^H value. The variety that has high titratable acidity decreases the pH value of the fruit, and it is not preferable for consumers consumption due to taste and other health issue such as dental erosion. Moreover, the second–best performer was the yellow capsicum, which had higher carotenoid compounds than the green variety. The variation of the biochemical compounds depends on different genetic make-up possessed in capsicum varieties. Keeping in mind the study's limitations, genetic diversity and gene bank can be established by investigating more varieties. Utilizing these data, the breeders could introduce newly developed varieties with desired genetic characteristics.

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