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RESEARCH ARTICLE

PREHARVEST ABIOTIC CONDITIONS ON THE ACCUMULATION OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITIES OF SWEET BELL PEPPER (*Capsicum annum* L) CULTIVARS IN THE LOW AND HIGH VELD REGIONS OF ZIMBABWE

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ABSTRACT

Abiotic factors coupled with varietal differences have a special bearing on the synthesis of bioactive compounds and enhancement of antioxidant capacities of sweet bell pepper. The aim of the present study was to characterize the content of bioactive compounds (lycopene, vitamin C, β-carotenes, total phenols, and the antioxidant activity of sweet bell pepper (*Capsicum annum* L) grown under different Agro climatic regions with different environmental conditions, the Eastern Highlands (High veld region) and the Save Valley (low veld region). The results from the study showed statistical differences ($p \leq 0.05$) between the different growing locations with regard to the bioactive compounds which were identified and quantified. *Capsicum annum* var. Lafayette presented the highest concentration of vitamin C content, lycopene content and total phenols as well as the greatest antioxidant activity in the high veld region. In addition, the results indicated that low temperatures favour *in vivo* biosynthesis of bioactive compounds and enhances antioxidant capacities of sweet bell pepper.

KEYWORDS

Antioxidant, Abiotic, Bioactive compound, High and Low veld regions.

1. INTRODUCTION

In the recent years people have developed interests in maintaining good health, therefore, the majority have become more careful in the food they choose to consume, looking for food with a high nutritional value, bioactive compounds and antioxidant capacity, such as fruits and vegetables. Epidemiological and nutritional studies have consistently demonstrated a positive relation between the consumption of fruits and vegetables (Vegetarian meals) and a reduction in the mortality rate due to heart disease, cancer, and other degenerative diseases, as well as the aging process (Gibson et al., 2010). This is due to the fact that these foodstuff compounds are the chief sources of nutraceutical compounds, such as vitamins, phenolic compounds, natural antioxidants, and other biotic compounds (Bayilli et al., 2011; Chávez-Mendoza et al., 2015). The sweet bell pepper (*Capsicum annum* L) is a fruit well known for its high content in bioactive compounds and strong antioxidant capacity and it is among the most popular of fresh vegetables worldwide due to its combination of colour, flavour, and nutritional value (Blanco-Ríos et al., 2013). Fresh sweet bell peppers have remarkably high quantities of ascorbic acid and their striking red colour is due to several carotenoid pigments that include β-carotene with pro-vitamin A activity and oxygenated carotenoids such as capsantine, capsorubin, and cryptocapsin, (Aditika et al., 2018) which are elite to these fruits and have proven to be operative at scavenging free radicals (Deepa et al., 2006). Peppers also contain huge quantities of neutral phenolic compounds or flavonoids named quercetin, luteolin, and capsaicinoids, (Chávez-Mendoza et al., 2015; Aditika et al., 2018).

The consumption of bioactive compounds offers beneficial properties in human health owing to their antioxidant properties, which protect against the oxidative damage to cells and thus avert the development of common degenerative diseases such as cancer, cardiovascular diseases, cataracts, diabetes, Alzheimer's, and Parkinson's (Blanco-Ríos et al., 2013). These chemical compounds also prevent the oxidation of essential fats within the cells of the brain that are considered necessary for its optimal functioning (Badawi et al., 2004; Agati et al., 2012). But under stress conditions (especially low temperatures) sweet bell peppers upsurge the generation of reactive oxygen species (ROS) in vivo usually in diverse cellular sections, including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (· OH) (Fini et al. 2011). These species can react with proteins, lipids and nucleic acids altering the biological potentiality of these biomolecules within the plant (Liu et al., 2008; Fini et al., 2011). In addition to this, plants are especially susceptible to the oxidative damage produced by ROS (Liu et al., 2008), hence the cellular oxidative damage plays a role in determining the relative efficiency of the cell function and, therefore, the behaviour of the crop under fluctuating environmental conditions towards production of the beneficial bioactive and antioxidant

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compounds. While biogenesis and functions of carotenoids are increasingly well described, the effect of environmental factors on the relevant signalling and metabolic pathways are far from being well understood (Apel and Hirt, 2004). Understanding the way environmental factors determine and influence carotenoid concentration represents an important objective and challenge for eco-physiologists and agronomists, but also for geneticists who need to understand better the interactions between environmental and genetic factors (Blokhina and Fagerstedt, 2010). Therefore, the main focus of this paper was to study the effect of preharvest abiotic conditions on the bioactive compounds and antioxidant capacities of Sweet bell pepper (*Capsicum annum* L) in Zimbabwe.

2. Materials and Methods

2.1 Growing Location

Capsicum cultivars were grown in the two distinct locations, namely Save Valley Research Station and Nyanga Research Station, a sister research experimental site. The former is located at an altitude of 435metres above sea level (a.b.s.l) (21°0'0" S and 31°30'0" E) and has a history of very few days of chilling conditions. The latter on the other hand lies at an altitude of 1679 meters above sea level (a.b.s.l) (18° 12' 36" S 32° 44' 24"E), which meant higher chance for chilling conditions. Chilling injury phenomenon that resulted into pitting symptoms was associated with average temperatures of 15^{0}C and below. Sweet bell pepper cultivars were harvested at commercial maturity stage (TSS = 5.68, L = 39.33, a = −17.09, b = 28.54) from the two locations on scheduled dates on a bi-weekly basis from late October 2019 up to mid-May 2020, which was the experimental period and the same run was done again in the October 2020 to February 2021 season. Four fruits were collected in each plot based on pitting symptoms commonly expressed as dots on the ripe fruit. If there was no sign of chilling injury, the samples were collected randomly.

2.2 Antioxidant Capacity

2.2.1 DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Activity Assay

Radical scavenging activity of sweet bell paper was determined by a revised method described by (Idris et al., 2017). A mixture of 2.5mL of DPPH solution (0.13mM) and 2.5mL of the sweet bell pepper extracts or standard drugs dissolved in methanol at varying concentrations of 0.005 to 0.08mg/mL was vortexed thoroughly and kept in the dark for 20 minutes. Absorbance of the mixture was spectrophotometrically measured at 517nm against the blank and control wells. The DPPH radical scavenging activity was calculated according to Iqbal et al., 2015, using the equation below:

$$(\%DPPH)\ Scavenging\ Activity = \frac{A_{DPPH-A_{extract}}}{A_{DPPH}} * 100$$

where A_{DPPH} = absorbance of the control, and $A_{extract}$ = absorbance of the extract (sample). The relationship between percentage inhibition and equivalent sample concentration was plotted to determine the half inhibitory concentration (IC_{50}) value of each sample.

2.2.2 ABTS$^+$ (2, 2` -Azino-Bis (3-Ethylbenzothiazoline)-6-Sulfonic acid) Radical Scavenging Activity

Oh et al., 2010, method was employed for the determination of ABTS$^+$ activity of sweet bell paper. Proportions of 1:1 for ABTS$^+$ (8mM) mixed with $K_2S_2O_8$ (2.55mM) were left in the dark for 15hours o enhance formation of a green coloured ABTS$^+$. The solution was then diluted with methanol (1:50v/v) to an absorbance of 0.800 ± 0.005 at 734nm which aided as the operational solution. Ten of the 1mL of the sample extracts and standard drugs (quercetin-3-rutinoside, known as rutin (flavanol), BHT, and gallic) at varying concentrations (0.0125 to 0.2mg/mL) was mixed with the ABTS$^+$ solution and was put in the a dark environment for eight minutes. Absorbance at 734nm was read against methanol (blank). The percentage inhibition of samples and standards was calculated using Iqbal et al., 2015, method employing the following equation:

$$(\%\ Inhibition)\ = \frac{Abs_{control-Abs_{sample}}}{Abs_{control}} * 100$$

where $Abs_{control}$ = absorbance of the control, and Abs_{sample} = absorbance of the extract (sample). The sample concentrations providing 50% (IC_{50}) of antioxidant activity were calculated from graph by plotting percentage inhibition of ABTS$^+$ by the samples against the corresponding sample's concentration.

2.3 β-Carotene

The method used for β-carotene determination was according to Naczk and Shahidi, (2004). The pulp of fresh sweet bell pepper was used for analysis. Sweet bell peppers were sliced into fine pieces. Samples of 10 g each were taken for preparation and were combined with a gram of magnesium carbonate, 10 g of sodium sulfate and 65 mL of tetrahydrofuran stabilized with 0.015% of butylated hydroxytoluene (BHT). The mixture was homogenized three times for 3 min and put on ice for 4 min to cool. The mixture was filtered and the residue was mixed with a second portion of 75 mL of tetrahydrofuran and the extraction was repeated again. Filtrates obtained were combined and evaporated using a Buchi rotary evaporator at 40 °C. The condensed volume obtained was made up to a specific volume of 25 mL with tetrahydrofuran to be analysed by HPLC. Quantification and identification was carried out using a Varian Prostar 320 HPLC system and a UV-Vis Prostar 210 detector and a C18-type column. The solvent system was of acetonitrile/tetrahydrofuran /water (85:12.5:2.5) pumped at a flow rate of 1 mL/min. All runs were at 24 °C and detection at 460 nm. HPLC peaks were identified using retention time comparison with trans-α-carotene (Type V) and β-carotene (Type IV) standards and internal standards. The limit of detection and limit of quantification of the analytical method used for β-carotene quantification were 0.03 and 0.10 µg/mL, respectively.

2.4 Determination of Bioactive Compounds

2.4.1 Lycopene

The lycopene content of bell pepper was determined conferring to the methodology by (Cucu et al., 2012). Sweet fresh bell pepper was milled and then, 1 g of sweet bell pepper was mixed for 2 min with 20 mL of buffer solution (hexane/acetone/ethanol 2:1:1) using an Ultra-Turrax on ice. The mixture which was obtained was then filtered and put into a separatory funnel, 50 mL of saturated NaCl solution was added and the solution was mixed for 2 min; the establishment of two phases was permitted and once phases were well-separated, the aqueous phase was discarded and the hexane phase recovered and filtered over anhydrous NaSO4 (5 g) which was rinsed three times with 2.5 mL of extraction buffer solution. The filtrate was dried up in nitrogen and the resulting residue was redissolved in tetrahydrofuran (THF) with butylated hydroxytoluene (BHT), and triethyl amide (TEA) at 0.05% to correct concentrations before being injected.

Quantification of lycopene content was done by HPLC, using a Varian ProStar 320 HPLC system with UV-Vis ProStar 210 detector (Prostar Varian Inc., Walnut Greek, CA, USA) at 472nm and a reversed-phase C18 column of 10 cm with MeOH/isopropyl alcohol/THF (30:30:35) comprising 250 ppm BHT and 0.05% TEA as the mobile phase. Flow rate was 1 mL/min, the temperature of the column was 35 °C, and the injection volume was 20 µL. The limit of detection and limit of quantification of the analytical method used for lycopene quantification were 0.01 and 0.02 µg/mL, respectively.

2.4.2 Vitamin C (Ascorbic acid)

Vitamin C content was determined by HPLC as described by (Doner and Hicks, 1981). The determination was done using fresh bell pepper which was harvested from the two aforementioned locations. The pepper pulp was cut into small pieces and ground to mash using a kitchen blender, then 10 g of bell pepper were then placed in 20 ml of an extraction mixture. Then, it was homogenized and filtered (using the Wattman filter paper) before being analysed. The extract was inserted into a Varian HPLC Prostar 320 equipped with a UV-Vis detector. An amine column Varian of 10 cm and a 20 µL injection loop were used. Limit of detection of the analytical process for vitamin C (ascorbic acid) quantification was 3.9 µg/mL. Linearity was determined from 0 to 250 µg/mL, using a standard vitamin C high purity reagent grade.

2.5 Total Polyphenol assay

The total phenolics of the extracts were quantified by the Folin and Ciocalteu reagent, following the modified method by (Singleton and Rossi,1965). Standard readings and sample extracts were prepared using

a spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian) at 765 nm against the reagent blank. The sample (test), (0.3 mL) was mixed with 0.6 mL of water and 0.2 mL of Folin-Ciocalteu phenol reagent. After 5 min, 1 mL of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 3 mL with double distilled water (DDW). The mixture was left in the dark for 30 min and after centrifuging the absorbance of blue colour from different samples was measured at 765 nm. Phenolic content was calculated as gallic acid equivalents GAE/g of dry plant material on the basis of a standard curve of gallic acid (5–500 mg/L, $Y = 0.0028x - 0.0056$, $R2 = 0.9998$).

2.6 Statistical Analysis

The data collected and quantified on the determination of total phenolics, lycopene, vitamin C and antioxidant properties using DPPH and ABTS+ was analysed using *R*-statistical software package version 4.0.1. The values are expressed as the mean ± standard deviation (SD). Analysis of variance and significance of difference among means were tested by one-way ANOVA and least significant difference (LSD) on mean values.

3. Results

3.1 Antioxidant Capacities

The use of DPPH was employed as a standard means for measuring the antioxidant capacity of sweet bell pepper elite varieties produced by Syngenta. The DPPH scavenging activities of sweet bell pepper cultivars are shown in Figure 1 below, and there were statistically significant ($p < 0.05$) interaction between region and the different cultivars of sweet bell pepper cultivars studied. Scavenging activity of DPPH free radicals measured as percentage inhibition ranged from 19% to 74% and from 16% to 58% in the High veld (Eastern highlands) and the Low veld regions (Save Valley region) of the different pepper cultivars, respectively. The highest antioxidant potential (scavenging activity) in the highveld and lowveld regions was observed in *Capsicum annum*. var Lafayette, being at par with the standard (quercetin-3-rutinoside) and it was concentration dependent. DPPH scavenging activities of the five sweet pepper cultivars in comparison to standard antioxidant (quercetin-3-rutinoside) showed a significant increase with regard to concentration. Antioxidant potential was the lowest in *Capsicum annum.* var. Balta in both the highveld and lowveld regions. The difference in the antioxidant activities reflected the nature and level of antioxidant compounds found in the sweet bell pepper plants in regard to geographical location and weather conditions during the growing period. The use of $ABTS^{+}$ was also employed as another way of determining antioxidant capacity in sweet bell pepper varieties. The $ABTS^{+}$ radical cation scavenging activity of different kinds of sweet bell pepper cultivars as shown in Figure 2, ranged from 12% to 69% and 7% to 58% in the highveld and lowveld regions respectively and among the *C. annuum* cultivars, Lafayette had the highest 3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^{+}$) scavenging capacity, while Balta had the lowest in all the sampling regions.

Free radical scavenging activity was assessed using DPPH and the $ABTS^{+}$ methods, which estimated antioxidant capacity as IC_{50} (Table 1). IC_{50} signifies the dose of sample that lessens by half the absorbance of a DPPH and a $ABTS^{+}$ reference solution therefore, a low IC_{50} designates a high antioxidant activity. The lowest radical scavenging activity (highest IC_{50} values) for both the DPPH and $ABTS^{+}$ was found in the high veld region (Eastern highlands) which has cold ambient conditions as compared to the low veld region (Save Valley).

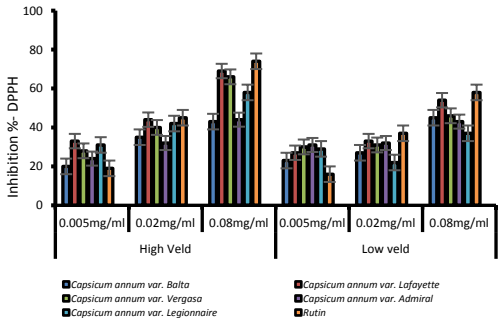

Figure 1: DPPH scavenging activity of ethanolic extract of the five varieties of Capsicum species. Values are mean ± SD of three replications.

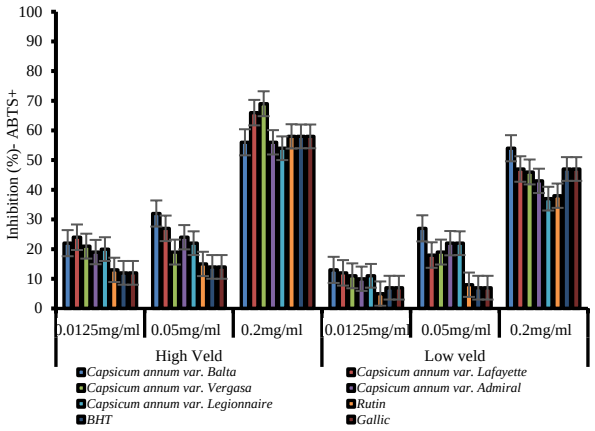

Figure 2: ABTS+ radical scavenging activity of ethanolic extract of the five cultivars of Capsicum species. Values are mean ± SD of three replications

Table 1: IC_{50} values of the varieties of Capsicum cultivar extracts and standard drugs.

Sample/standard	DPPH		ABTS+	
	Low veld	High veld	Low veld	High veld
Capsicum annum var. Balta	0.0797	0.0792	0.0055	0.0033
Capsicum annum var. Lafayette	0.0779	0.0662	0.0066	0.0023
Capsicum annum var. Vergasa	> 0.08	0.0523	0.0048	0.0039
Capsicum annum var. Admiral	> 0.08	0.060	0.0059	0.0019
Capsicum annum var. Legionnaire	> 0.08	> 0.08	0.0028	0.0043
Rutin	0.0057	0.0058	0.0055	0.0034
Gallic	-	-	0.0043	0.0046
BHT	-	-	0.0058	0.0074

3.2 β carotene

The statistical analysis for β carotene content in the five different varieties of sweet bell pepper showed statistically significant differences ($p \leq 0.05$) in the two different regions. On average, a greater content in β-carotenes was found in the peppers harvested in the highveld region, with the highest ($9000\mu g/100gFW$) content in β carotene found in *Capsicum annum* var. Lafayette, followed by *Capsicum annum* var. Legionnaire ($8500\mu g/100gFW$), with the least β-carotene content found in *Capsicum annum* var. Balta ($5000\mu g/100gFW$) under the highveld sampling region. For the Lowveld region, the trend in β-carotene content had *Capsicum annum* var. Lafayette ($6000\mu g/100gFW$) > *Capsicum annum* var. Legionnaire ($5500\mu g/100gFW$) > *Capsicum annum* var. Vergasa ($5000\mu g/100gFW$) > *Capsicum annum* var. Balta ($4500\mu g/100gFW$) with the least content found in *Capsicum annum* var. Admiral ($4000\mu g/100gFW$). The results found in this study were lower than reported by (Brikis et al., 2018), in pepper varieties cultivated both in the conventional and organic systems.

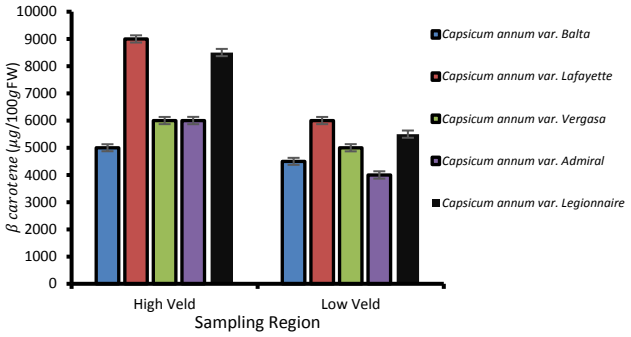

Figure 3: β carotene content in five different sweet bell pepper varieties harvested on two different sampling regions. Values are mean ± SD of three replications.

3.3 Lycopene and Vitamin C content

Figure 4 shows the content in vitamin C found in sweet bell pepper harvested in two sampling areas. The statistical analysis revealed differences ($p \leq 0.05$) between cultivars but not between sampling points ($p \geq 0.05$). The vitamin C (ascorbic acid mg/100g FW) content in the cultivars analysed from the highest to lowest followed the following order: *Capsicum annum*. var Lafayette (160mg/100gFW) > *Capsicum annum*. var. Legionnaire (145mg/100gFW) > *Capsicum annum*. var Vergasa (140mg/100gFW) > *Capsicum annum*. var Balta (120mg/100gFW), with the lowest of all being *Capsicum annum*. var Admiral (115mg/100gFW) but being statistically comparable to the last three cultivars in the highveld region. This trend in terms of vitamin C content of sweet bell pepper cultivars was similar to that which was found in the low veld region, serve only for the small content differences on averaging.

On average *Capsicum annum*. var. Lafayette had a 1.5-fold higher content in vitamin C compared to all the other cultivars in the different regions under study. By contrast, *Capsicum annum*. var Admiral had a lower vitamin C concentration, with an average content of 107.5 mg/100 g fresh weight. These figures are lower than those found by Iqbal et al., (2015), in grafted bell pepper cultivars and higher than those reported by Brikis et al. (2018), in the bell pepper cultivated under open field conditions. Bell peppers are known to be an excellent source of vitamin C. All the varieties analysed in the present study proved to be excellent sources of vitamin C and exceeded the recommended daily dosage (60 mg/100 g). The identification of dietary sources of vitamin C is imperative since humans do not have the ability to synthesize vitamin C due to a series of mutations of the gene encoding gulonolactone oxidase which catalyses the last enzymatic step in ascorbate synthesis.

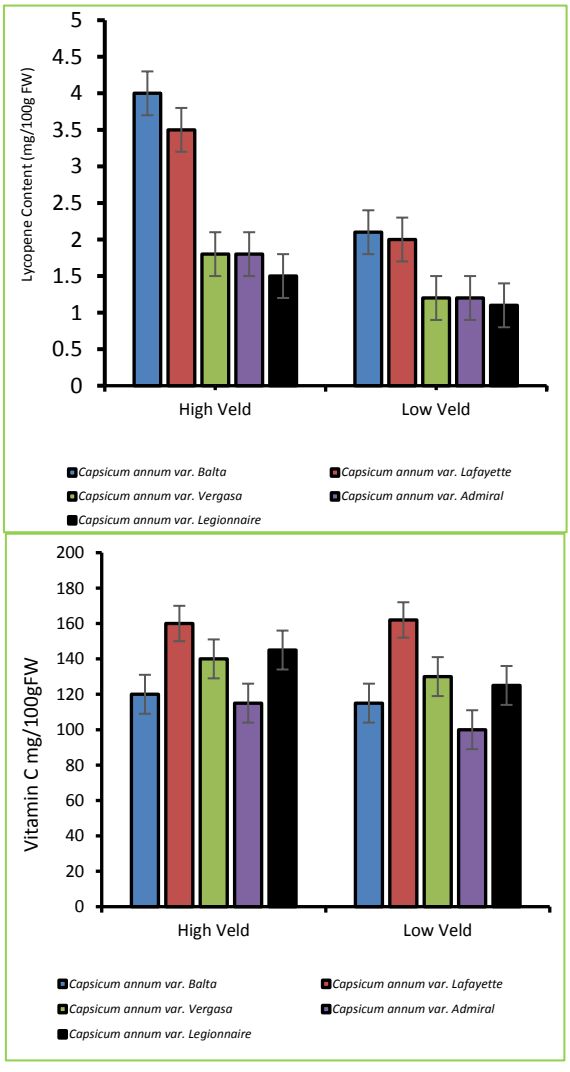

Figure 4: Lycopene and Vitamin C content ($\mu g/100 g$FW) of the five cultivars of Capsicum species. Values are mean ± SD of three replications.

3.4 Total Polyphenol

The statistical analysis for the total polyphenol content of sweet bell pepper cultivars indicated significant differences ($p \leq 0.05$) among the two distinct growing regions. High levels of total phenols were found in the high veld (Eastern highlands) sample extracts as compared to the low veld samples. *Capsicum annum*. var Lafayette presented the highest value of total phenols (66%) in the high veld region and *Capsicum annum*. var. Legionnaire was the lowest. Under the lowveld region, the total polyphenol content was quite comparable with slight differences in content which was not statistically significant amongst the varieties. It is well known that content of phytochemicals, including phenolic compounds present in vegetables, is affected by agronomic conditions as well as growing region due to differences in ambient production conditions (Rivero et al., 2001). Among the phytochemical compounds, polyphenols are of particular interest due to their property of scavenging free radicals in vivo. Epidemiological studies have shown a possible association between the consumption of polyphenols and a lower risk of coronary disease and cancer in people.

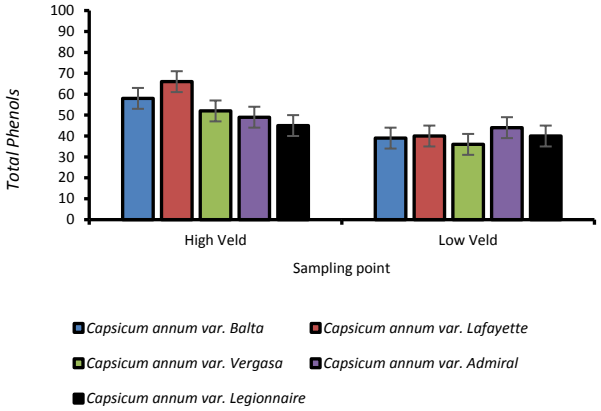

Figure 5: Total Polyphenol content of the five cultivars of Capsicum species. Values are mean ± SD of three replications

4. Discussion

Differences in the antioxidant capacities and bioactive compounds of the sweet bell pepper grown in the two distinct geographical locations were determined by the cultivar (Ghasemnezhad et al., 2011; Zhuang et al., 2012) as well as environmental factors (Simmone et al., 1997). Genetics seems to play a key role in the capability of cultivars to respond to biotic and abiotic stresses and are eventually responsible for the cultivars overall phytochemical content and antioxidant capacity, (Gifford et al., 2013). Bioactive and antioxidant activities of sweet bell pepper accumulated in high amounts in the high veld region (Eastern Highlands) and this was favoured mostly due to low temperatures that are experienced in the region as compared to the low veld region which is a bit cooler. Cold stress exposure for the Capsicum varieties grown was enhanced by almost double the vitamin C levels, lycopene content and also the antioxidant capacities of these varieties and this is also in agreement with (Yoon et al., 2017), for bell pepper which was grown aeroponically under chilling environments. Watanabe and Ayugase, (2015), found that the nutritional quality of winter sweet spinach (*S. oleracea* L.) was higher if the crop was subjected to low temperatures, considering the abundance in ascorbic acid (vitamin C), lycopene content and total phenols resulting from chilling stress. Sweet bell pepper plants grown under the lowveld region showed an increase of ascorbic acid (vitamin C) and this was in agreement with, (Rivero et al., 2001; Sun et al., 2007).

The phenolic concentrations in heat-stressed plants can have different behaviours depending on the species and their tolerance or sensitivity to high temperatures. Low temperature exposure of pepper plants increased the accumulation of total polyphenol content compared to normal temperature as was articulated by (Cucu et al., 2012) in tomato bioactive compounds studies. As reported by (Oh et al., 2010), the total phenolic content increased in response to chilling (4°C for 1 day) in lettuce plants (*Lactuca sativa* L.) grown in a growth chamber, this is in agreement with the results that were obtained from this study on the total phenolic

content of sweet bell pepper which had high polyphenol content from the Eastern Highlands which had low chilling temperatures. Phenolic compounds are important for the appearance, taste and aroma of food products, as well as for their health-promoting aspects (Colla et al., 2013; Chen et al., 2013), through their most pronounced scavenging activities of the compounds.

Low temperatures have been seen to induce the accumulation of 4-aminobutyrate and 4-hydroxybutyrate, (Chandra et al., 2014). These metabolites seem to be correlated to multiple abiotic stresses and play an important role in the regulation of transcriptional and biochemical mechanisms, which lead to the accumulation of bioactive compounds and antioxidant capacities of horticultural crops in particular sweet bell pepper.

5. Conclusions

Sweet bell pepper succumbs to many abiotic stresses of seasonal occurrence that influences plant growth and productivity. Abiotic stresses can be employed as tools to enhance the nutraceutical quality of sweet bell peppers. However, the effects of practical implementation can differ depending on factors such as genetic diversity, agronomical practices and environmental ambient conditions. The enhancement of health-related properties of bioactive and antioxidant compounds due to abiotic stresses should be obtained without affecting the yield. For these reasons, understanding the mechanisms adopted for plants to counteract these stresses is the key step to control the abiotic stresses and use them as tools to improve the properties of crops in as far as production of bioactive and antioxidant capacities of sweet bell pepper is concerned.

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