DISSERTATION

ESTIMATION OF CAPSAICINOID COMPOUNDS AND OTHER NUTRITIONALLY IMPORTANT COMPOUNDS IN COLORADO GROWN PEPPER CULTIVARS

Submitted by

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ABSTRACT

ESTIMATION OF CAPSAICINOID COMPOUNDS AND OTHER NUTRITIONALLY IMPORTANT COMPOUNDS IN COLORADO GROWN PEPPER CULTIVARS

Peppers (*Capsicum annum L.)* are an important crop usually consumed as food or spice. Peppers contain a wide range of phytochemicals such as capsaicinoids, phenolics, anthocyanins, carotenoids, and vitamin C . However, capsaicinoids are the major group of compounds that give them their characteristic pungent taste. Capsaicinoid compounds have been used in the food and pharmaceutical industries because of their potential antioxidant, anticancer, and antibiotic properties. The first project (chapter 2) evaluated the capsaicinoid compounds and other bioactive compounds in fresh and roasted pepper cultivars. In addition, the effect of roasting on their nutritional content was investigated. Samples in this study were collected from Colorado State University's Arkansas Valley Research Center in Rocky Ford (AVRC) at different pepper pod stages. Capsaicin and dihydrocapsaicin were quantified using a Waters HPLC system equipped with a fluorescence detector for the capsaicinoid compounds. The levels of capsaicin were in the range of 0 – 3636 µg/g in the green stage and 0- 4831 µg/g in the red stage in the selected peppers with the highest levels in Habanero and the lowest level in Sweet Delilah. Scoville Heat Units (SHU) were in the range of 0 - 112,588, helping to determine which peppers could be classified as hot, mild, and sweet. Capsaicinoids were not detected in sweet pepper varieties such as Flavorburst, Canario, Sweet Delilah, and Aristotle. There was a significant difference in the levels of capsaicinoid compounds after the roasting method in all pepper cultivars. The TF content of the pepper cultivars ranged from 204.44 (Habanero) to 756 (Flavorburst) µ g/g in the green stage and

from 557.28 (CSU 321) to 962.71 (Numex Joe E Parker) µg/g in the red stage.

Both raw and roasted peppers possessed strong antioxidant activity as determined by 2,2-diphenyl-1-picrylhydrazyl) reagent (DPPH, 61–87%) and 2,20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS, 73–159 µg/g) assays.

The highest antioxidant potential in the green and red stages was observed in Canario. There was a reduction of antioxidant activity in cooked peppers in both the DPPH and ABTS assays. Ascorbic acid and antioxidant activity decreased after roasting in the mature green and red stages, whereas total phenolics and flavonoids increased except in the mature green stage of Sweet Delilah and yellow stage of Canario.

Peppers are a perishable commodity and have a limited shelf life. Therefore, the (chapter 3) objective of this project (chapter 3) was to evaluate the effect of storage time, packaging, and the 1-MCP on weight loss, firmness, respiration rate, ethylene production, ascorbic acid, and antioxidant activity, and bioactive compounds of Sweet Delilah pepper. Four packaging films were tested in this study, Polypropylene (P12F), Laminated Poly-Nylon (30NV), Coextruded Vacuum Pouch (30NVC), and polyethylene (P15G) with different thicknesses. The packaged peppers showed the lowest reduction of weight loss compared to the control. During the red stage's storage time, firmness loss was 13% in peppers treated with 1-MCP, whereas 25% loss in firmness in control samples. The results indicate that the respiration rates and the ethylene production significantly decreased as storage time increased in packaged peppers compared to control samples. At the end of storage time, the highest respiration rate was in control samples, whereas the lowest rate of respiration rate was in packaged peppers with (P12F) films. The range of total phenolic and total flavonoid compounds were 3782, 5090, and 519, 646.84 µg/g, respectively, in the green and red stages.

Peppers treated with 1-MCP- maintained high levels of phenolic and flavonoid compounds compared with control samples. Moreover, the results showed that the highest phenolic and flavonoid loss was in control samples, while the lowest phenolic and flavonoid loss was in packaged peppers. The highest ABTS activity was 150 µmol TE/g in when packaged P12G films, whereas the lowest ABTS activity was 143.20 µmol TE/g in control samples in the red stage. Peppers packaged with films retained ascorbic acid levels than other peppers packaged with other films and control samples.

Peppers are rich source of bioactive compounds such as phenolics, flavonoids, carotenoids, and capsaicinoid compounds. These compounds have been shown various health benefits effect on human health. Therefore, these compounds must be bioavailable to achieve their health beneficial effects. The bioaccessibility of total phenolics, total flavonoids, total carotenoids, and capsaicinoid compounds in different cooked potatoes mixed with two roasted peppers, Joe Parker (hot) and Sweet Delilah (sweet), were investigated using an in-vitro method. In this (chapter 4) the objective was to identify differences in the bioaccessibility of bioactive compounds among potato cultivars (Purple Majesty (PM), Yukon Gold (YG), and Rio Grande Russet (RG)) and a numbered line (CO 97226-2R/R (R/R) combined with roasted pepper varieties. The bioactive compounds and capsaicinoid compounds in potatoes and peppers were estimated before and after the digestion. Our results indicated that 53 - 75% of phenolic compounds content was released from potatoes cultivars mixed hot roasted pepper, whereas 53.4-88% in potatoes varieties mixed sweet roasted pepper respectively. The highest level of carotenoids was 194.34 µg/g in YG and 42.92 µg/g in the RG cultivar when mixed with roasted Joe Parker pepper (JP). No capsaicinoid compounds were detected in potato cultivars mixed with roasted Sweet Delilah pepper (SD).

After in-vitro digestion, a significant reduction was observed in bioactive compounds.

Based on the results of the included studies, the bioaccessible amount of total phenolics ranged from 485-252 µg/g in potato cultivars mixed with hot roasted peppers. The bioaccessible amount of flavonoids ranged from 185.1-59.25 µg/g. The results indicated that the YG cultivar mixed with JP and SD showed the highest phenolics and carotenoids bioaccessibility. In contrast, the PM mixed with JP and SD the lowest phenolics and carotenoids bioaccessibility. Our results indicated that the highest flavonoid bioaccessibility showed in R/R mixed with hot and sweet roasted peppers. The lowest flavonoids bioaccessibility showed in PM and the RG. Additionally, the capsaicinoid bioaccessibility was studied in different potato cultivars mixed with roasted peppers. The maximum bioaccessible amount of capsaicin was observed in YG mixed with JP, while the minimum bioaccessibility was observed with PM.

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CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1-Introduction

Peppers are one of the most commercially important crops that grow in tropical regions around the world (González-Zamora et al., 2013; Park et al., 2012). Pepper is herbaceous plant of the Capsicum genus belonging to the Solanaceae family, including many other important commercial crops such as tomato, potato, and eggplant (Al Othman et al., 2011; Gebhardt., 2016; Bayogan et al., 2017). The genus Capsicum comprises many wild and cultivated species, and five species of peppers grown commercially are including *Capsicum annuum, C. baccatum, C. chinense, C. frutescens, and C. pubescens* (Bae et al., 2012; Asnin and Park., 2015; Thuphairo et al., 2019; Zhuang et al., 2012). Furthermore, pepper fruits have been typically classified as non-climacteric fruits, there is a huge diversity of pepper cultivars on the market varying in shape, color, and taste (Ilić et al., 2012; Palma et al., 2014).

Pepper is a crop like tomato, eggplant, corn, cucumber, and melons that require specific conditions for growing. In the USA, peppers are widely grown all over the United States for local consumption. Pepper fruits are mainly produced in the field on raised beds using the drip irrigation system (Biswas et al., 2017). According to USDA, in 2019, the total planted area of bell peppers in the USA was 39,200 acres, while the total planted area of chile peppers was 10.600 acres (USDA, 2019). Furthermore, the total harvested acreage of bell peppers in the USA slightly decreased from 42,200 acres in 2017 to 38,300 acres in 2019. The total harvested acreage decreased from 16,000 acres in 2017 to 10,200 acres in 2019. The total production volume of bell peppers in the USA slightly decreased from 14,390.0 cwt in 2017 to 12,134.5 cwt in 2019, whereas

the total production volume of chile peppers decreased from 3,336.0 cwt in 2017 to 1,644.0 cwt in 2019 (USDA, 2019). In 2019, the value of utilized production of vegetable crops was $14.2 billion, while the value of utilized production of bell pepper 557,660 million and 63,711 million of chile peppers (USDA 2019). Furthermore, from a global perspective, the USA ranked 6th in production of green peppers both chile and bell peppers between 2007 and 2011 with approximately 3% of reported world production (USDA 2013).

Recently, the consumption of peppers has increased, mainly due to the high potential of their bioactive compounds. Pepper fruits can provide an important number of bioactive compounds such as phenolics, flavonoids, and carotenoids to the human diet (Ornelas-Paz et al., 2010; Zhuang et al., 2012; Sora et al., 2015). These bioactive compounds are known to add health promoting benefits to the diet (Lillywhite et al., 2013; Chávez-Mendoza et al., 2015). Interestingly, pepper fruits can be consumed at different ripening stages (green, yellow, and red) either with salads or as side dishes (Abbie et al., 2005). In the USA, the per capita use of bell peppers remained relatively steady through 2000 and 2001 at 7.4 and 7.5 pounds per person, respectively but increased slightly in 2010 and 2012 (9.5 and 10.7 pounds per person, respectively), reaching the maximum consumption in 2019 (11.3 pounds per person) (Shahbandeh 2020). Due to the combination of color, taste, and nutritional value, peppers are the most popular fresh vegetables in the world. They have been used as a color, flavor, and preserve foods, as well as for medicinal purposes (Blanco-Ríos et al., 2013; Chopan et al., 2017). The attractive colors of pepper fruits are due to carotenoid pigments that include β-carotene and oxygenated carotenoids such as capsanthin, capsorubin, and cryptocapsin (Chávez-Mendoza et al., 2015; Hassan et al., 2019).

Pepper fruits are an excellent source of phytochemical compounds such as capsaicinoid, polyphenolics (Bae et al., 2012; Campos et al., 2013). Capsaicinoid compounds are the major group of alkaloid compounds responsible for the pungency taste of hot pepper varieties (Viktorija et al., 2014; Barbero et al., 2014). Besides the capsaicinoids compound, pepper fruits contain a varied range of phytochemical compounds such as carotenoids, anthocyanins, and vitamin C (O'Sullivan et al., 2010; Caporaso et al., 2013). Due to their antioxidant activities, anticancer and antibiotic properties, these compounds have been used in the food technologies and pharmaceutical industries (Hervert-Hernández et al., 2010; Sora et al., 2015). The levels of these compounds are increased in the advanced stage of ripening due to the degradation of chlorophylls and increase the enzymatic activity during the ripening process. The concentrations of these bioactive compounds among pepper varieties are influenced by various factors, including environmental conditions, stress factors, postharvest storage conditions, genotype, and ripening stages of the fruits (Alvarez-Parrilla et al., 2011; Ghasemnezhad et al., 2011).

Pepper fruits are harvested at different maturity stages requiring specific conditions for maintaing commercial quality as long as possible (Tsegay et al., 2013). Pepper fruits are highly perishable, the pepper quality is affected by numerous factors such as postharvest handling, transportation, storage time, and marketing conditions (Tan et al., 2012; Manolopoulou et al., 2010; Mahajan et al., 2016). Pepper fruits are not suitable for long-term, several factors cause pepper fruit losses, including increased respiration rate, hormone production such as ethylene, physiological disorders, and senescence (Chitravathi et al., 2016). Moreover, pepper fruits are requiring optimal postharvest technologies to maintain their storage stability and extend their shelf life during storage time. Due to chilling injury, pepper fruits cannot be stored at low temperatures. Therefore, to maintain a high quality of pepper fruits it is essential to control the temperature and

relative humidity during the storage time (Ilić et al., 2012). Studies have suggested that optimum storage temperature and high relative humidity may slow down the water loss and increase the shelf life of peppers fruits (Sharma et al., 2013). For instance, pepper fruits can be more susceptible to physiological and pathological deterioration when stored in optima storage conditions (Malik et al., 2016; Sharma et al., 2018). The storage life of pepper fruits is limited by many factors such as water loss, shriveling, tissue softening, physiological disorders, and fungal infection (Ščetar et al., 2010;). Several technologies have been applied to increase the shelf life of vegetables and fruits during storage time. These techniques are used for pepper fruits to reduce water loss, delay the ripening process, chilling injury symptoms, and therefore to extend the shelf life.

Peppers fruits have a wide range of bioactive compounds that provide various health benefits such as, anti-inflammatory, antimicrobial activities, protecting against hypercholesterolemia, and atherosclerotic cardiovascular diseases (Campos et al., 2013; Caporaso et al., 2013; Chávez-Mendoza et al., 2015). Therefore, the bioactive compounds in our daily diet must be bioavailable to achieve beneficial effects (Rein et al., 2013; Pugliese et al., 2013; Thakur et al., 2020). Furthermore, bioactive compounds are present at high concentrations in pepper fruits; however, the bioaccessibility of these can be highly variable. Although various studies have been conducted on the bioactive compound in pepper fruits, only limited information is available regarding their bioaccessibilities. Several studies have demonstrated that the bioaccessibility of bioactive compounds depends on factors such as food matrix, digestion conditions, uptake, interaction with other dietary ingredients, cooking methods, and metabolism reactions (Actis-Goretta et al., 2013; Thakur et al., 2020). It is important to note that the most common bioactive compounds in the human diet have different bioaccessibility. The bioaccessibility of bioactive compounds can be defined as the part of the compound released from the food matrix and becomes

available for absorption (Buggenhout et al., 2010; Tagliazucchi et al., 2010; Pugliese et al., 2013). The overall objectives of this dissertation are: -

1-To estimate the capsaicinoid compounds, polyphenols, and the antioxidant activities of *Capsicum annuum* and to investigate the changes in phytonutrients after the roasting method.

2-To evaluate the effect of different packaging films and 1-Methylcyclopropane application on peppers quality parameters, i.e., weight loss, firmness, respiration rates, bioactive compounds, antioxidant activities, and ascorbic acid during storage.

3-To investigate the bioaccessibility of bioactive compounds, phenolics, flavonoids, carotenoids, and capsaicinoids in cooked potato cultivars mixed with different roasted pepper varieties in *in-vitro* digestion experiment.

2. LITERATURE REVIEW

2.1 Capsaicinoid compounds

Capsaicinoids are the most abundant group of alkaloids compounds and one of the many phytochemicals that pepper contains (Bley et al., 2012). The level of capsaicinoids determines whether the pepper is hot, milled, or sweet. Along with capsaicinoids, peppers also encompass flavonoids, flavanols, phenolics, and vitamins (Liu et al., 2010; Caporaso et al., 2013; Fattori et al., 2016). Due to their pungency properties, capsaicinoid compounds have been used commonly in food products as spice or food additives. According to previous studies, pepper verities contain varying concentrations of capsaicinoid compounds. The primary capsaicinoid in chili pepper is capsaicin and dihydrocapsaicin. They represent more than 90% of the total capsaicinoids content in hot varieties (Reyes-Escogido et al., 2011; Orellana-Escobedo et al., 2013). Besides capsaicin and dihydrocapsaicin, other minor capsaicinoid compounds found in chile peppers include nor-dihydrocapsaicin and homo-capsaicin homo-dihydrocapsaicin Figure1 (Barbero et al., 2014; Fattori et al., 2016). Studies have showed that capsaicinoid compounds are synthesized in the epidermal cells of the placenta tissues. Capsaicinoid compounds are much higher in the placenta than in the pericarp of the pepper fruits (Ornelas-Paz et al., 2010). Capsaicin (trans-8-methyl-N-vanillyl-6 nonenamide) is the main capsaicinoid compound in hot pepper varieties and its major parameters determine its commercial quality and the levels of pungency (Bley et al 2012; González-Zamora et al., 2013). Capsaicin is a crystalline and lipophilic compound with the molecular formula $C_{18}H_{27}NO_3$ and molecular weight 305.40 g/mol (Reyes-Escogido et al., 2011).

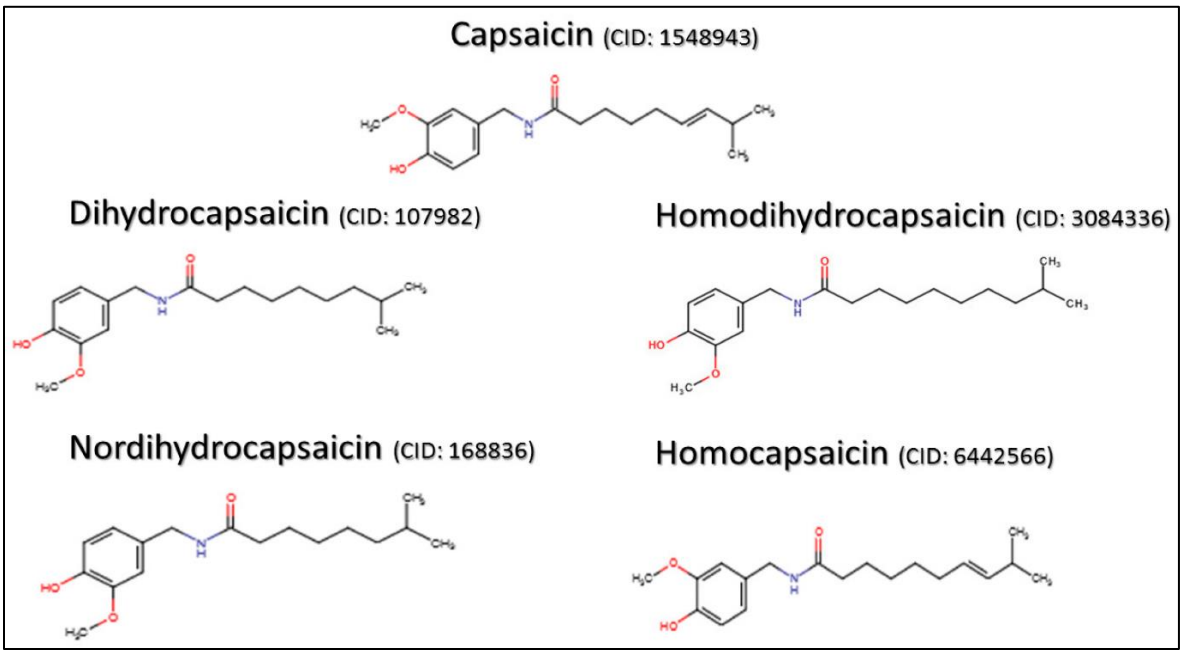


Figure 1.1 The chemical structure of capsaicinoid compounds (Fattori et al., 2016).

Capsaicinoids are synthesized naturally from valine and phenylalanine by enzymatic condensation and different-sized fatty acid chains which are elongated by a fatty acid synthase (Reyes-Escogido et al., 2011 González-Zamora et al., 2013). They have three regions in their structure, aromatic ring containing a OH- group, an amide bond, and a hydrophobic side (Figure 1.1). Two pathways involved in the biosynthesis of capsaicinoid compounds: fatty acid metabolism and the phenylpropanoid pathway. The first pathway determines the phenolic structure, and the second pathway determines fatty acid molecule's fatty acids (Figure 1.2).

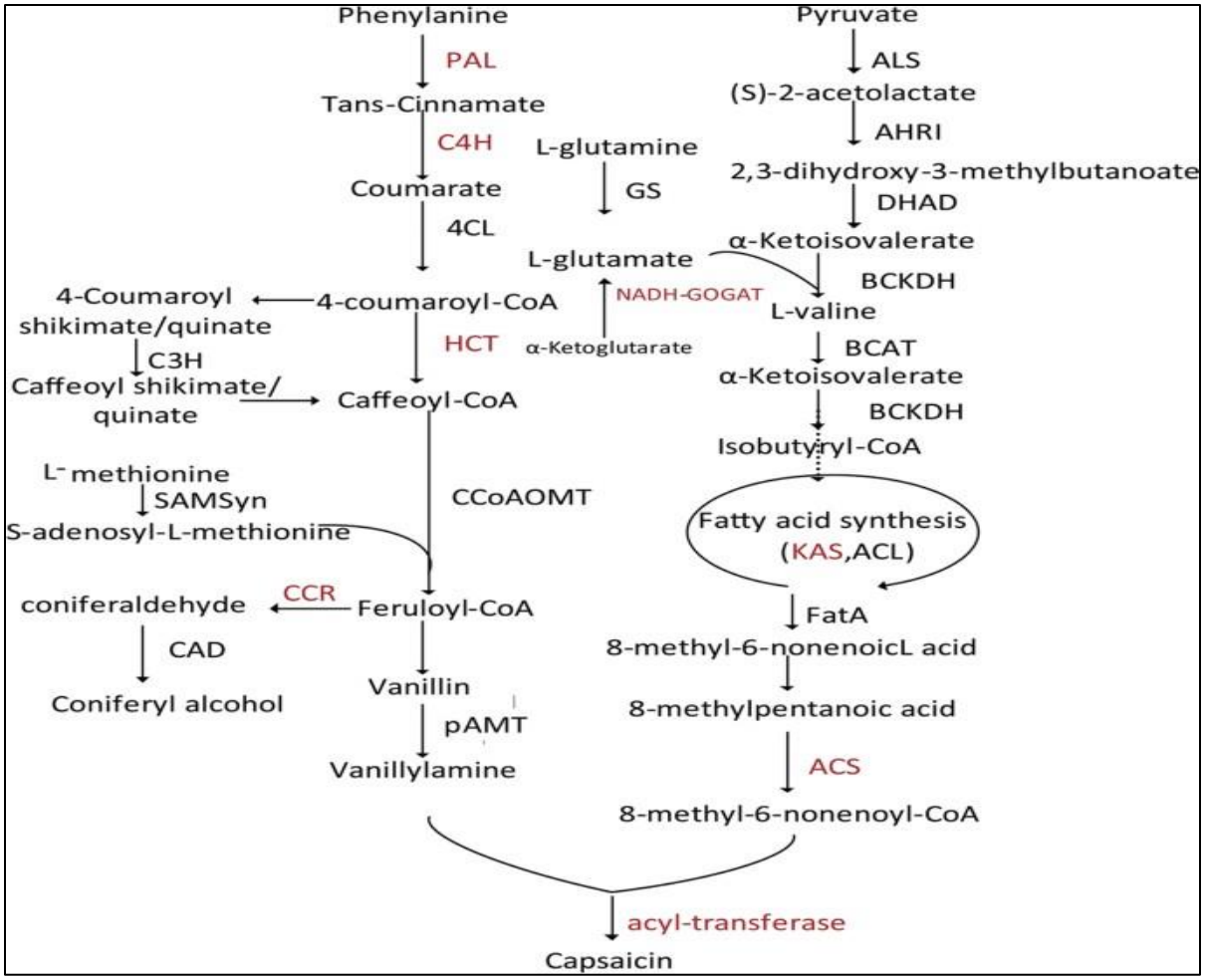


Figure 1.2 The capsaicin synthesis pathways (Zhang et al., 2016).

Several studies suggested that capsaicinoid compounds have pharmacological and physiological effects on the gastrointestinal tract, cardiovascular and respiratory system (Al Othman et al., 2011). In various applications, capsaicin has been reported to show protective effects against high cholesterol levels and obesity. Due to their pharmacological properties, capsaicin can be used as a topical cream to treat neuralgia, musculoskeletal pain, diabetic neuropathy, osteoarthritis, and rheumatoid arthritis (Korkutata and Kavaz 2015). Furthermore, capsaicinoid compounds have been shown beneficial health effects such as analgesia, anticancer, anti-inflammatory, antioxidant, and anti-obesity activities (Liu et al., 2010; Huang et al., 2013).

Due to antioxidant properties, capsaicinoid compounds play an important role in protecting cellular systems from oxidative damage (Park et al., 2012; Zheng et al., 2017).

The concentrations of capsaicinoid compounds in pepper fruits depend on various factors, including varieties, maturation stages, growing conditions, and climate (Hwang et al., 2012; Zhuang et al., 2012; Aza-Gonzalez et al., 2011). In the early stages of fruit development, the capsaicinoid compounds increase until reaching a maximum level. Then, the levels of these compounds decrease with the fruit development due to the activity of peroxidase enzyme (Reyes-Escogido et al., 2011; Barbero et al., 2014). Different methods are used to quantify the capsaicin and dihydrocapsaicin in peppers. The concentrations of capsaicinoid compounds in hot peppers can be calculated by Scoville Heat Units (SHU) (Nadeem et al., 2013). There are five degrees of pungency by using Scoville heat units (SHU): non-pungent (0–700 SHU), mildly pungent (700–3,000 SHU), moderately pungent (3,000–25,000 SHU), highly pungent (25,000–70,000 SHU) and very highly pungent (>80,000 SHU) (Al Othman et al 2011). Several studies suggest that the diversity of capsaicinoid compounds has been shown in different pepper varieties (González-Zamora et al., 2013). A study carried out by Ornelas-Paz et al. (2010) that the Mexican raw peppers are rich in capsaicinoids, and in raw peppers the concentration of capsaicin is (0.6–913.8 μg/g), while dihydrocapsaicin (0–756.9 μg/g).

The maximum concentration of capsaicinoid compounds in the Cayenne pepper (*Capsicum annuum L.*) was 1789 μmol/Kg FW during peppers fruits development (Barbero et al., 2014). Huge variability in the capsaicinoid contents was observed. The capsaicin level was ranged from 1.24-746.80 μg/g FW, while the dihydrocapsaicin level was ranged from 55.56 – 496.08 μg/g FW (Zhuang et al., 2012). Similar observations were made by Alvarez-Parrilla et al., (2011) the total capsaicinoid content in fresh Jalapeno˜ and Serrano were ranged from 78.65 to 386.38 μg g/g. In

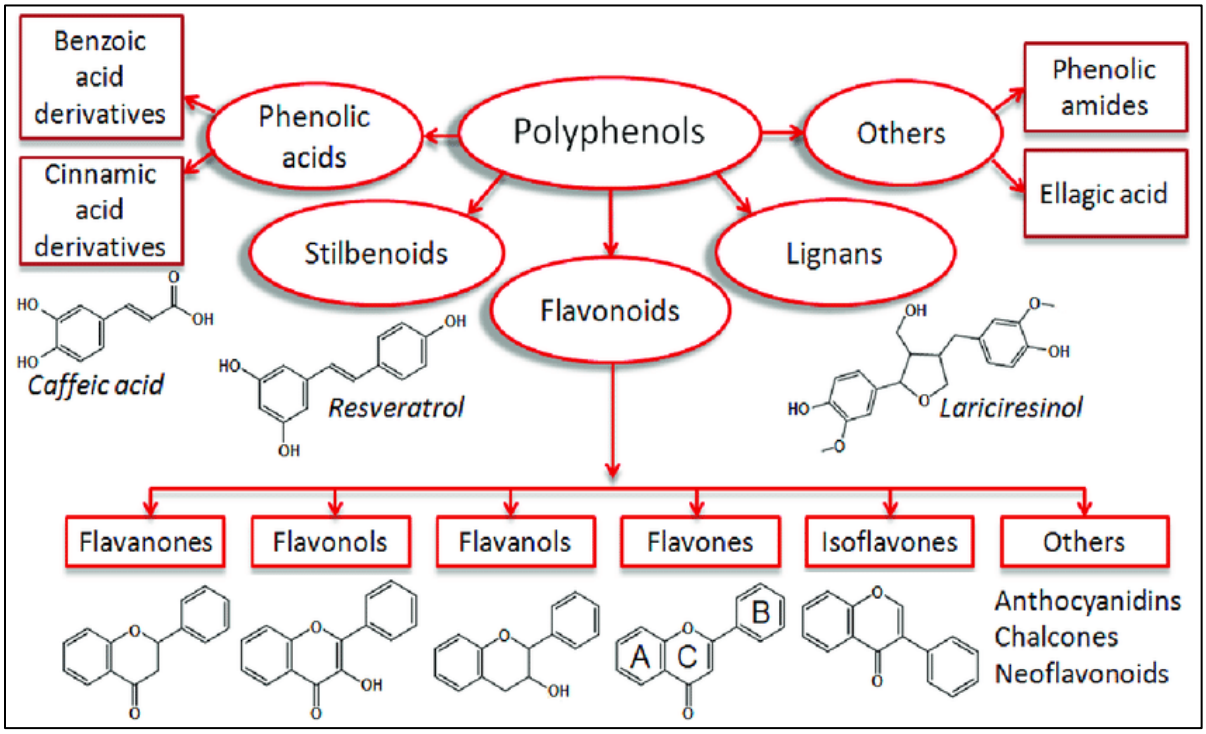
a study conducted by Korkutata and Kavaz (2015), the total capsaicinoid compounds in five hot peppers (*Capsicum annum L.*) were ranged from 72.30 ± 21.6 to 128.40 ± 42.3 mg/kg. Pepper verities can be classified as highly pungent as the Scoville Heat Unit (SHU) values, thus the highest concentration of capsaicin was 9.177 ± 0.268 mg/g, whereas the lowest concentration of capsaicin was 1.189 ± 0.073mg/g in yellow peppers varieties (Nwokem et al., 2010).

There was a significant difference in capsaicin and dihydrocapsaicin in chili pepper genotypes using ultra-fast liquid chromatography. It ranged from 0–13,076 μg/kg and 0–7,155 μg/kg for both capsaicin and dihydrocapsaicin, respectively (Usman et al., 2014). Victoria-Campos et al, (2015) noted that the total capsaicinoid compounds ranged from 1057.9 and 2294.6 μg/g in fresh green and red Jalapeño peppers, respectively and the level of total capsaicinoid content was higher in fresh red peppers than in green peppers. The difference of capsaicinoid compounds in pepper fruits could be attributed to the difference in fatty acids available for capsaicin biosynthesis (Aza-Gonzalez et al., 2011).

2.2 Polyphenolics compounds

Polyphenolics, secondary plant metabolites, constitute the largest groups of phytochemical compounds that are beneficial to human health, mainly due to their antioxidant properties (Arnnok et al., 2012; Medina-Juárez et al., 2012; Scalbert et al., 2011; Campos et al., 2013). In addition, phenolics contribute to the taste, color, and nutritional value of many vegetables and fruits. There is a wide diversity of phenolics in vegetables and fruits (Blanco-Ríos et al., 2013; Iqbal et al 2015). Phenolics are complex of organic substances, which contain more than one phenolic group and there is large variability in the levels of polyphenolics in vegetables and fruits (Bayili et al., 2011; Campos et al., 2013). Phenolics are produced in the shikimic acid and pentose phosphate pathways

through phenylpropanoid route. According to their structure, phenolic acids can be divided into two subgroups: the hydroxybenzoic and the hydroxycinnamic acids.



Figuer 1.3 diagram of polyphenol compounds (Dirimanov et al., 2019).

Polyphenols play an important role in the protection of plants against plant feeding insects and herbivores (Buer et al., 2010; Mierziak et al., 2014). Besides their importance for plants, polyphenolics are important for human health as antioxidant agent due to their ability to inhibit and prevent the free radical compounds such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Park et al., 2012; Wang et al., 2018). The potential activity of phenolic compounds is based on the redox properties of their hydroxyl groups in their chemical structure (Oboh and Rocha., 2007; Mierziak et al., 2014). The polyphenols content is varying among pepper varieties and the concentration depends on the varieties, maturity stage, growth conditions, and

geographical origin. For instance, the level of the total polyphenol content of the hot pepper fruits increased from 2138.1 GAE/kg^{-1} FW in green stage to 7915.7 GAE/kg^{-1} FW in red stage (Maria et al., 2018). Mexican raw peppers are also rich in phenolic compounds, and the total phenolics in raw pungent peppers were ranged from1150.5 to 2190 μg/g GAE (Ornelas-Paz et al., 2010).

In study carried out by Arnnok et al. (2012) the total phenolics in hot chili pepper (*Capsicum annuum L.*) were ranged from 0.796–4.70 g GAE kg^{-1}. The total phenolic contents were ranged from 19.21 to 28.43 and 21.81 to 37.64 mg GAE/g DW in fresh and boiled peppers, respectively (Shaimaa et al., 2016). The highest level of total phenolics was 14.80 mg GAE/g DW in green varieties, while the lowest level of phenolics was 12.35 mg GAE/g DW in orange varieties (Blanco-Ríos et al., 2013). Also, Sora et al. (2015) reported that the phenolic contents of the peppers (pulp and seed) ranged from 119.97 ± 3.44 to 2060.12 ± 20.56 mg GAE/100 g. These differences could be explained by quantification methods, diversity of varieties and genotypes, and maturity stage (Hervert-Hernández et al., 2010). The increase in phenolic compounds could be attributed to the ability of a plant to acclimate and stimulate these compounds under biotic or abiotic stress (Isah et al., 2019).

Flavonoids, a large class of polyphenols compounds in plants, thus these compounds provide flavor and color in fruits and vegetables (Agati et al., 2012; Kanazawa et al.,2012; Mierziak et al., 2014). There are subclasses of flavonoid compounds such as flavones, flavanones, flavanols, anthocyanidins, and isoflavones (Bae et al., 2012; Perla et al., 2012). Flavonoid compounds have been shown high antioxidant activities and anticancer activities (Bae et al., 2012; Shaimaa et al., 2016). The concentrations of these compounds depend on several factors such as varieties, maturation stages, growth conditions (Ghasemnezhad et al., 2011; Vera et al., 2017). The total flavonoids of green and red pepper were 7.8, 4.1 and 10.4 mg QE/100 g FW, respectively

(Lin and Tang 2007). The highest total flavonoids were 60.36 ±9.94 mg QE/100g FW in (Caribe) variety, while the lowest level of total flavonoids was 25.38 ±3.44 in Anaheim variety (Medina-Juárez et al., 2012). The total flavonoids in different sweet bell peppers were 7.53, 4.80, 4.26, and 4.27 mg CE/g DW in green, orange red, yellow varieties, respectively (Blanco-Ríos et al., 2013). The highest level of flavonoid was 441 mg CE/100 g DW in the Serrano variety, while the lowest level of total flavonoid was 201 mg CE/100 g DW in the Meoqui variety (Alvarez-Parrilla et al., 2011). In a study conducted by Thuphairo et al. (2019), the different colored sweet peppers contained different levels of flavonoids, including quercetin 71.71-102.33 μg/g dw and luteolin 56.34-95.89μg/g dw. The yellow peppers had higher concentrations of quercetin than other colored peppers.

2.3 Ascorbic acid

Peppers fruits are a rich source of antioxidants compounds such as vitamin C, vitamin E, and carotenoids (Zhang and Hamauzu., 2003; Bicikliski et al., 2018). Due to its health promoting effects, vitamin C is one of the most essential phytochemical compounds in pepper fruits (Korkutata and Kavaz., 2015; Figueroa-Méndez and Rivas-Arancibia., 2015). Several studies suggested that vitamin C plays an essential role in chelating heavy metal ions, scavenging free radical compounds, and suppressing peroxidation (Tan et al., 2012; Patrick et al., 2016; Nerdy., 2018). The levels of vitamin C in vegetables and fruits can be influenced by several factors such as types of cultivars, climatic conditions, maturity stage, harvesting methods (Bicikliski et al., 2018). Various studies suggested that the level of ascorbic acid in fruits is associated with carbohydrate metabolism. The level of ascorbic acid is high in ripening fruits due to the conversion of sugars (Martínez et al., 2005). The higher levels of vitamin C found at advanced stages of ripening might be related to increased glucose levels, which is the precursor of vitamin C

biosynthesis (Ornelas-Paz et al., 2013). The levels of ascorbic acid were ranged from 584 mg AA/100 g DW in Serrano to 2153 mg AA/100 g DW for in Jalapeño (Alvarez-Parrilla et al., 2011). There was a significant difference in the levels of ascorbic acid in pepper varieties and the level of ascorbic acid was ranged from 121.14 to 251.60 mg/100g FW (Medina-Juárez et al., 2012).

In study carried out by Teodoro et al. (2013), it was reported that vitamin C contents in Habanero pepper accessions (*Capsicum chinense*) ranged from 54.1 to 129.8 mg/100g. Nerdy (2018) reported that a significant difference in the content of vitamin C among different bell peppers. For instance, the highest level of ascorbic acid was 1.74 mg/g DW in green varieties, while the lowest level of ascorbic acid was 0.49 mg/g DW in orange varieties (Blanco-Ríos et al., 2013). The quantity differences in levels of vitamin C among the varieties could be attributed to several factors, including soil, climate, growing conditions, cultivar, production practices. The level of vitamin C decreases in advanced ripening stages due to the degradation of vitamin C by peroxidase enzymes (Mendoza et al., 2015).

2.4 Antioxidant Activities

Bioactive compounds in peppers have been reported to possess several biochemical and pharmacological properties such as antioxidant, anti-inflammatory, anti-allergic, and anticarcinogenic activities (Ozgur et al., 2011; Hwang et al., 2012; Zhuang et al., 2012) Antioxidant activity describes the ability of redox molecules in foods and biological systems to scavenge free radical compounds such as Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Alvarez-Parrilla et al., 2011; Kalita and Jayanty., 2014). Interestingly, the antioxidant activity of bioactive compounds is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors, single oxygen quenchers, and metal chelators

(Deepa et al., 2007; Lobo et al., 2010; Zhuang et al., 2012). These compounds can delay or inhibit cellular damage through their free radical scavenging property (Lobo et al., 2010; Martí et al., 2011).

Due to their beneficial effects, several methods have been developed to study free radical scavenging antioxidant activity. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays are the most popular radicals for measuring antioxidant activity of bioactive compounds. Sora et al., 2015 reported that the ABTS method yielded better antioxidant values than the DPPH assay. As the health promoting capacities of pepper fruits depend on type of varieties, maturation stage, and food processing. Several studies reported that antioxidant activity of peppers increased due to increasing the levels of bioactive compounds in the maturation stage (Ghasemnezhad et al., 2011; Nadeem et al., 2013). Medina-Juárez et al. (2012) reported that Bell and Caribe varieties had the highest DPPH activity, while Serrano, Anaheim and Jalapeno varieties had the lowest DPPH activity. In a study carried out by Zhang and Hamauzu (2003), the red pepper variety showed a higher level of DPPH radical scavenging activity compared to green and yellow pepper varieties. Similar observations were made by Blanco-Ríos et al. (2013) that the red pepper extract showed the highest antioxidant activity, while the orange pepper extract showed the lowest antioxidant activity. Medina-Juárez et al. (2012) reported that Bell and Caribe varieties showed higher antioxidant activity (34.44 ±0.43 and 33.60 ±1.35 μM ET/g FW, respectively. The trolox equivalent antioxidant capacity in habanero pepper (*Capsicum chinense*) genotypes was ranged from 1.55 to 3.23 mM/mg (Campos et al., 2013). The variation in total antioxidant activity between Chiltepin (*Capsicum annuum*) and Habanero (*Capsicum chinense*) peppers may be attributed to several factors such as fertilization process, fruit maturity, and temperature (Gonzalez-Mendoza et al., 2012). The variation of

antioxidant activity in pepper varieties could be attributed to the differences in carotenoid, phenolic, and flavonoid contents in peppers varieties (Zhang and Hamauzu 2003; Medina-Juárez et al., 2012; Park et al., 2012; Alvarez-Parrilla et al., 2013; Vera et al., 2017).

3. Effect of cooking methods on bioactive compounds

3.1 Capsaicinoid compounds

Peppers can be eaten raw or cooked in different ways such as roasting and boiling. Cooking methods have been shown a significant impact on the content of bioactive compounds and antioxidant activities in several vegetables and fruits. A few studies have been conducted to study the effects of cooking on the capsaicinoid compounds in pepper fruits. In some of these studies, the impact of cooking methods on the bioactive compounds are contradicting with some reported increase, while others reported a decrease in the bioactive compounds in several vegetables and fruits.

The stability of capsaicinoid compounds can be influenced by several factors, including processing and storage conditions. For instance, the level of capsaicinoids in paprika was significantly decreased as storage time increased, and the maximum decrease was in dihydrocapsaicin (Topuz et al., 2004). The highest loss of capsaicin in red pepper (*Capsicum annuum*) was ranged from 18% to 36% after boiling, while the maximum loss was observed after pressure cooking method (Suresh et al., 2007). Similarly, the levels of both capsaicin and dihydrocapsaicin in chili pepper were decreased after heating for 15 min at 100 °C (Wang et al., 2009). Several studies suggested that heat treatment of vegetables results in greater liberation of bioactive compounds (Lemmens et al., 2010). The impact of heat processing on the capsaicinoid concentration depends on the capsaicinoid type and ripening stage. For instance, the grilling caused

significant increases in capsaicin (6.1–924.9%), dihydrocapsaicin (2.6–57%) and nor-dihydrocapsaicin (6.6–206.8%) (Ornelas-Paz et al., 2010). Similarly, the lowest concentration of total capsaicinoids was (1057.9 μg/g dry weight) in fresh green, while the highest level of total capsaicinoids (3538.3 μg/g dry weight) in grilled green pepper (Victoria-Campos et al., 2015). The capsaicin content in dried pepper was 4 to 10 times higher than in fresh peppers (Popelka et al., 2017). In a study conducted by Toontom et al., (2012), the drying methods increase the level of capsaicinoid compounds, thus the highest level of capsaicin was in dried pepper fruits compared to fresh samples. The increases of capsaicinoid compounds by cooking methods could be attributed to dehydration of food matrices, improved extractability, liberation of conjugated capsaicinoids, and inactivation of capsaicinoids destroying enzymes such as peroxidases (Schweiggert et al., 2006; Ornelas-Paz et al., 2010; Lemmens et al., 2010; Victoria-Campos et al., 2015).

3.2 Polyphenolics

The effect of heat processing on polyphenolic compounds in vegetables and fruits has been studied, and cooking methods can both decrease or increase the levels of bioactive compounds including polyphenolics (Ornelas-Paz et al., 2010). However, all the three used cooking methods led to increasing the total phenolics in the following order: microwaving > boiling > steaming > fresh (Turkmen et al., 2005). Ornelas-Paz et al. (2010) reported that the boiling caused an increase in total phenolic content in all pungent peppers from 1745.9 to 2549.7μg GAE/g FW, whereas the total phenolic content was decreased in non-pungent peppers. Shaimaa et al. (2016) reported that the total phenolic contents in sweet and peppers ranged from 19.21 to 28.43 and 21.81 to 37.64 mg GAE/g DW in fresh and boiled peppers, respectively, indicating that the boiling treatment increased the total phenolic contents. The total phenolic compounds in dried chili extract was

significantly increased after roasting (Muangkote et al., 2019). These changes can be ascribed to dehydration of food matrix and inactivate the polyphenol oxidase enzyme during cooking, leading to the inhibition of polyphenol degradation (Schieber et al., 2006; Victoria-Campos et al., 2015; Montoya-Ballesteros et al., 2014; Minatel et al., 2018; Gunathilake et al 2018). In addition, Turkman et al. (2005) reported increase in total phenolics in several vegetables due to the disruption of cell walls, which liberate soluble phenolic compounds from insoluble ester bonds. The increase in phenolic compounds during cooking methods could be attributed to the breakdown and hydrolysis of the complex polyphenolic compounds such as tannins to simple polyphenols (Gunathilake et al., 2018; Buratti et al., 2020). On the other hand, various studies indicated that the degradation of the polyphenolic compounds depends on their chemical structure of these compounds in the fruit or vegetable (López-García et al 2018). The highest reduction of total phenolics in red peppers was observed after boiling, followed by steaming and roasting (Hwang et al., 2012). These results were like those reported by El-Hamzy et al., (2016) that the total phenolics levels of the red Jalapeno slices were decreased after drying methods. The reduction in the levels of polyphenols, flavonoids, and flavonols has been attributed to the selective leaching of these compounds from commodity during the cooking methods (Perla et al., 2012). Reduced polyphenolic compounds during cooking methods has been ascribed to thermal damage of polyphenolic compounds, and the leaching of polyphenolic compounds into the cooking water (Doymaz et al., 2002; López-García et al., 2018).

3.3 Ascorbic acid

Peppers are excellent sources of antioxidant compounds such as β-carotene, vitamins E, K, and ascorbic acid (Ghasemnezhad et al., 2011; Park et al., 2012; Teodoro et al., 2013). The

concentrations of these compounds in our daily diet have been affected by cooking methods such as boiling and roasting. Ascorbic acid is extremely sensitive and unstable in response to the oxidation process. Therefore, it is easy to lose by a varied range of oxidizing agents such the high temperature, high intensity of light, metal content, and the higher the activity of the ascorbate oxidase enzyme (Oyetade et al., 2012; Diengdoh et al., 2015; Singh and Harshal., 2016).

According to previous studies, the level of vitamin C decreased during cooking methods, and there are significant differences in the levels of ascorbic acid before and after heat processing. In this regard, the cooking methods such as roasting lead to a substantial loss in ascorbic acid compared with fresh peppers (Hwang et al., 2012). The content of vitamin C in red chili pepper was found to be reduced to 90% after drying at high temperatures (Montoya-Ballesteros et al., 2014). The levels of ascorbic acid were ranged from 306 to 3438 μg/g in raw peppers, while the level of ascorbic acid was reduced 15–87% after heat treatments (Ornelas-Paz et al., 2013). The reduction of vitamin C during heat processing has been attributed to that vitamin C is unstable at high temperatures. Peppers showed the highest loss of vitamin C, while Croat showed the lowest loss of vitamin C after heat treatments (Igwemmar et al., 2013). The levels of ascorbic acid in all dried peppers varied between 14.21 and 51.55 mg/100g whereas, the ascorbic acid level of fresh pepper was 53.19 mg/100g (Toontom et al., 2012). A similar result was reported by (Hwang et al., 2012). Several studies reported that vitamin C is highly sensitive to oxidation, thus vitamin C can be destroyed rapidly after harvesting or during storage (Balan et al., 2016). Finally, the losses of vitamin C are influenced by the cultivar, the stage of maturity, the cooking temperature, and the duration of cooking.

3.4 Antioxidant activity

Similar to polyphenols and ascorbic acid, the antioxidant activity of vegetables and fruits is affected by cooking methods. Cooking methods such as boiling, microwaving, and roasting could cause a high loss of bioactive compounds, resulting in reduced antioxidant activities. The DPPH radical scavenging activity was 117.82 mg AA eq/100g in raw red pepper, whereas the DPPH radical scavenging activity was decreased by 46.56-68.29, 82.10-90.10, 99.25-112.44, and 99.68-104.15 mg AA eq/100 g for boiling, steaming, stir-frying, and roasting, respectively (Hwang et al., 2012). Ornelas-Paz et al. (2013) have reported that boiling and grilling caused a 6–93% reduction in the antiradical activity of pungent peppers. A higher reduction in the antioxidant activity of red pepper was observed in boiling and steaming than stir-frying and roasting (Hwang et al., 2012). The redaction of antioxidant activity has been ascribed to loss of ascorbic acid and polyphenols because of the dissolving of these compounds into the cooking water (Perla et al., 2012; Victoria-Campos et al., 2015).

On the other hand, several studies have shown that different cooking methods can improve the antioxidant capacity of some vegetables due to the release of phytochemical compounds from the food matrix (Turkmen et al., 2005; Ornelas-Paz et al., 2013). Turkmen et al. (2005), who studied the effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables, found that the antioxidant activity of pepper, green beans, broccoli, and spinach significantly ($p < 0.05$) increased during different cooking methods compared to fresh values. In a study carried out by Shotorbani et al. (2013) that the various temperatures influenced the antioxidant activity of sweet bell pepper phenolic extracts and therefore the scavenging activity of DPPH radical of red and gijlar pepper extract was increased at 50 °C and 65 °C, respectively. The roasting method at 90 °C for 25 min increased the antioxidant activity of dried peppers (Muangkote

et al., 2019). El-Hamzy et al. (2016) have studied the effect of different drying methods on antioxidant activity in red Jalapeno pepper. They found that the antioxidant activities of all dried samples were higher than in the fresh samples.

4. Effect of packaging films and 1- MCP on the quality parameters and bioactive compounds.

Pepper fruits are an excellent source of micronutrients, and phytochemical compounds and these compounds should be preserved during the storage time of pepper fruits (Alvarez-Parrilla et al., 2011; Tsegay et al., 2013; Hameed et al., 2013). Reducing the respiration rate, delaying the ripening and senescence processes are the most important factors in maintaining a high quality of bioactive compounds and extending the shelf life of pepper fruits. The quality of pepper fruits during storage time depends on the temperature and relative humidity of storage (Samira et al., 2013). Therefore, controlling temperature, relative humidity, and as well as the use of chemical preservatives can extend the shelf life of pepper fruits during storage time (Ilic et al., 2017). The shelf life of pepper fruits depends on various factors such as production time, quality, storage conditions, and handling methods (Manolopoulou et al., 2010). Several studies indicated that softening, shrinkage, wilting, and pathogenic disorders are the most common issues of pepper fruits, which reduce the quality and acceptability of peppers (Rao et al., 2011; Mahajan et al., 2016; Sharma et al., 2018). Recently, various techniques such as packaging films and chemical applications have been used to extend the shelf life and storability of perishable commodities (Manolopoulou et al., 2012). Packaging films, one of the most important techniques that have been successfully used to prevent decay in various vegetables and fruits (Sahoo et al., 2014), and chemical treatments can delay physiological processes such as water loss, respiration rate, transpiration, ethylene production, and softening (Ilic et al., 2017; Barbosa et al., 2020). Therefore, polyethylene and polypropylene are the most common packaging films that have been used for

extending the shelf life of fresh pepper fruits.

Several studies suggested that use packaging films can create modified atmospheric conditions around the product, thus reducing the respiration rate, transpiration, and other metabolic processes (Chitravathi et al., 2015; Soltani et al., 2016). Sahoo et al. (2014) have studied the effect of packaging materials low-density polyethylene (LDPE) and polypropylene (PP) on the shelf life of bell pepper. They found that the maximum weight loss was in control, unpackaged pepper samples, while the minimum loss of weight was in packaged ones. Also, a study carried out by Sharma et al. (2018) exhibited that control samples had the maximum loss in weight, followed by the peppers packaged in two different packaging materials and stored at different storage temperatures. The low weight loss could be attributed to the reduction of physiological processes such as respiration and transpiration (Edusei et al., 2012). In addition, firmness is one of the most important factors determining the quality of vegetables and fruits (Požrl et al., 2010). Peppers stored in packaging films showed the lowest reduction of firmness, while the control samples showed the highest reduction of firmness (Manolopoulou et al., 2012). These results are in accordance with those reported by Ornelas-Paz et al. (2015) who reported that the firmness of the packed peppers was significantly higher than that of the unpackaged peppers. Pepper fruits stored in polypropylene bags were higher firmness than those in polyethylene bags (Shehata et al., 2013). These data agree with another study reported by Mahajan et al., (2016) that pepper fruits packed in shrink packaging film maintained the highest average firmness, while the control fruits registered the lowest mean of firmness under SMC. Similarly, green pepper packaged exhibited a lower flesh softening and cell wall disassembly during low-temperature storage (Chitravathi et al., 2016). A high reduction of firmness could be explained by the increase of transpiration rate in pepper fruits.

The respiration rate is one of the most important physiological processes that determined the shelf life of pepper fruits. A significant difference in the respiration rate was observed between peppers packaged with different materials and control samples of green chili (*Capsicum annum L.)* during storage at 8 ± 1 °C (RH 85–95%). Therefore, the respiration rate decreased significantly with the increase in storage period (Chitravathi et al., 2016). Interestingly, these results are in accordance with those reported by Manolopoulou et al., (2012) who, observed that the levels of O_2 were decreased in packaged peppers, whereas the levels of CO_2 were increased during storage time. There is little published information on the effects of packaging on the bioactive compounds and their antioxidant activity in peppers. The total phenolic was decreased during storage time in both polyethylene and jute bags, and the lowest reduction was 14.1% in polyethylene, while the highest reduction was 22.8% in jute bags (Iqbal et al., 2015). On the other hand, the content of total phenolic compounds increased in the unpacked peppers that had been stored at 23 °C, and then the content decreased (Ornelas-Paz et al., 2015).

The highest reduction of ascorbic acid was noted in control samples (unpacked) during storage time as compared with packaged pepper fruits (Manolopoulou et al., 2012). During storage time of dry, hot peppers, the reduction of ascorbic acid was 87.8%, 77.9% and 72.4% at 20 °C, 25 °C and 30 °C in polyethylene bags, while 85.3%, 73.8% and 66.8% at 20 °C, 25 °C and 30 °C in jute bags (Iqbal et al., 2015). Antioxidant compounds can delay or inhibit the oxidation or free radical-mediated oxidation of a substrate. In the study carried out by Chitravathi et al. (2016), the authors found that chilies packaging maintained pigment stability, lower loss of phenolic compound, and lower reduction of ascorbic acid as compared with control unpackaged samples (Dhall et al., 2013; Grzegorzewska et al., 2020). During storage, ethylene production can cause both an increase in respiration rate and color changes in several vegetables and fruits, and studies

have demonstrated that ethylene exhibits both beneficial and deleterious effects on produce. Promotion of senescence, fruit softening, and discoloration are examples of deleterious effects of ethylene. Therefore, controlling ethylene production during storage time may extend the shelf life of pepper fruits. Several studies reported that physical, chemical, and gaseous treatments have been applied to maintain the quality of products with high nutritional value (Mahajan et al., 2010; Lima et al., 2015). Ethylene production can be inhibited by some chemical inhibitors such as 2-aminoethoxyvinyl glycine (AVG), silver ions (Ag), and the gaseous compound 1-methylcyclopropene (Schaller and Binder., 2017). 1-MCP one of the most common methods used to reduce ethylene production in several fruits and vegetables (Thakur et al., 2017). The application of 1-MCP has been shown to delay the ripening process by slowing respiration rate, lower lipoxygenase activities, delayed color changes, and inhibiting ethylene production (Ilić et al., 2012; Relox et al., 2015). 1-MCP acts as an ethylene inhibitor. It binds to ethylene receptors in the plant cell and prevents ethylene from binding, thereby inhibiting ethylene signal transduction (Alabboud et al., 2017).

Several studies suggested that vegetables and fruits treated with 1-MCP had better quality and storability than untreated vegetables and fruits. The red pepper fruits treated with 1-MCP showed less ethylene production compared with the untreated fruits (Fernández-Trujillo et al., 2009). Cao et al. (2012) reported that 1-MCP treated peppers had lower respiration rates and ethylene production than control fruits. Huang et al. (2003) have demonstrated that pepper fruits treated with 1-MCP delayed color loss, fruit softening and extended the storage life of pepper fruits by inhibiting ethylene production. Similar observations were made by Ilic et al. (2012) that the green peppers treated with 1-MCP had a significant effect on delaying ripening processes, inhibiting color changes, decreasing decay of pepper fruits. In study conducted by Tan et al.,

(2012) pepper fruits treated with 1-MCP maintained high levels of phenolic compounds and high antioxidant activities.

6. The bioaccessibility of bioactive compounds in peppers.

Pepper fruits comprise various phytochemical compounds such as capsaicinoids, phenolics, flavonoids, and carotenoids, which are highly desirable in our daily diet (Álvarez-Parrilla et al., 2011). Due to the presence of the phytochemical compounds, peppers fruits have been shown various health benefits effect on human health (Alvarez-Parrilla et al., 2011; Shotorbani et al., 2013). Therefore, these compounds must be bioavailable to achieve their health beneficial effects (Thakur et al., 2020). Although research indicates that the bioactive compounds provide health benefits effects, a few studies have been published on the bioaccessibility of bioactive compounds in pepper fruits. Furthermore, studies have demonstrated that the bioaccessibility of bioactive compounds depends on various factors, such as physicochemical properties, food matrix, heat processing, preservation methods, and interactions with other compounds (Tydeman et al., 2010; Actis-Goretta et al., 2013; Thakur et al., 2020). The bioaccessibility of bioactive compounds can be analyzed by *in-vitro* methods.

The in-vitro methods are one of the most reliable, accurate, and frequently employed methods for estimating the bioaccessibility of bioactive compounds (Thakur et al 2020). A few studies have investigated the bioaccessibility of polyphenolic and capsaicinoid compounds in pepper fruits. In a study conducted by Hervert-Hernández et al., (2010), who found that 75% of total polyphenols amount released from the food matrix by the action of digestive enzymes. The bioaccessibility of capsaicin in fresh green peppers was 120%, while the bioaccessibility of dihydrocapsaicin was 150% in fresh green peppers, respectively (Victoria-Campos et al., 2015). The bioaccessibility of bioactive compounds depends on several factors such as physical properties

of the food matrix, genotype, chemical structure of carotenoids, polarity and solubility of carotenoids, food processing, and potential susceptibility of carotenoids (Andre et al., 2015; Hervert-Hernández et al., 2010). Moreover, carotenoids' bioaccessibility in red peppers ranged from 48.0 to 97.0% (Granado-Lorencio et al., 2007). A similar observation was made by Aherne et al., (2010) that the carotenoid bioaccessibility varied within and between the three pepper varieties, and the bioaccessibility of carotenoids from peppers ranged from 6.2% to 100%. Different hot peppers' bioaccessibility values ranged from 33 to 49% for β-carotene, from 20 to 41% for β-cryptoxanthin, and from 25 to 49% for zeaxanthin, respectively (Hervert-Hernández et al., 2010). The capsanthin and zeaxanthin of red chili peppers *(Capsicum annuum)* had the highest bioaccessibility, while b-cryptoxanthin, violaxanthin, and b-carotene had the lower bioaccessibility (Pugliese et al., 2013). Additionally, the bioaccessibility of lutein and zeaxanthin in the yellow potato ranged from 76 to 82% for lutein and from 24 to 55% for zeaxanthin (Andre et al., 2015). It was observed that thermal processes and dietary fat could improve the bioaccessibility of bioactive compounds. Therefore, there was a significant variation in the bioaccessibility of bioactive compounds during heat processing. Cooking methods can influence the bioaccessibility of bioactive compounds, mainly through changes in the plant cell wall structure and properties (Rein et al., 2013; Thakur et al., 2020). Therefore, the impact of heat processing on the capsaicinoid bioaccessibility depends on the capsaicinoid type and ripening stage. For instance, the bioaccessibility of dihydrocapsaicin was improved after boiling, while the bioaccessibility of capsaicin was improved after grilling (Victoria-Campos et al., 2015).

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CHAPTER 2: CAPSAICINOIDS, POLYPHENOLS, AND ANTIOXIDANT ACTIVITIES OF CAPSICUM ANNUM: COMPARATIVE STUDY OF THE EFFECT OF RIPENING STAGE AND COOKING METHODS.

Summary

Peppers (*Capsicum annuum L.*) are an important crop usually consumed as food or spices. Peppers contain a wide range of phytochemicals, such as capsaicinoids, phenolics, ascorbic acid, and carotenoids. Capsaicinoids impart the characteristic pungent taste. The study analyzed capsaicinoids and other bioactive compounds in different pepper cultivars at both the mature green and red stages. The effect of roasting on their nutritional content was also investigated. In the cultivars tested, the levels of capsaicin ranged from 0 to 3636 µg/g in the mature green stage and from 0 to4820 µg/g in the red/yellow stage. The concentration of dihydrocapsaicin ranged from 0 to 2148 µg/gin the mature green stage and from 0 to 2162 µg/g in the red/yellow stage. The highest levels of capsaicin and dihydrocapsaicin were found in the Habanero, whereas the lowest levels of capsaicin and dihydrocapsaicin were found in the Serrano variety. The levels of capsaicinoid compounds in mature green and red /yellow stages were either reduced or increased after roasting depending on the cultivar. The ranges of total phenolic and total flavonoids compounds were 2096 to7689, and 204 to 962 µg/g, respectively, in the green and red/yellow mature stage pods. Ascorbic acid levels in the peppers ranged from 223 to 1025 mg/ 100 g Dry Weight (DW). Both raw and roasted peppers possessed strong antioxidant activity as determined by 2,2-diphenyl-1-picrylhydrazyl) reagent (DPPH, 61–87%) and 2,20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS, 73–159 µg/g) assays. Ascorbic acid and antioxidant activity decreased after roasting in the mature green and red stages, whereas total phenolics and flavonoids increased except in the mature green stage of Sweet Delilah and yellow stage of Canrio.

1. Introduction

Peppers are one of the most widely consumed food. They have diverse flavors, culinary uses, and nutritional content. After being introduced from the Americas, peppers have been incorporated into cultures and cuisines globally. Besides their direct culinary uses, peppers are also used for coloring, flavoring, preserving, nutraceutical, and medicinal purposes. Peppers belong to the genus Capsicum. *C. annuum, C. frutescens, C. chinense, C. pubescenes,* and C. *baccatum* are grown domestically or commercially (Taylor et al., 1975; Ramchiary et al., 2008) Of these C. annuum is grown most extensively.

Peppers are an excellent source of phytochemicals, such as anthocyanins, vitamins, phenolic acids, flavonoids, carotenoids, and capsaicinoids (Kumar et al., 2009; Howard et al., 2000). Various studies have demonstrated the benefits of bioactive compounds of peppers in vitro and in vivo. These compounds provide many nutritional and health benefits that include antioxidant, anti-inflammatory, and antimicrobial activities reduced prevalence of type 2 diabetes and obesity, protection against hypercholesterolemia, and reduced prevalence of atherosclerotic cardiovascular diseases (Spiller et al., 2008; Alvarez-Parrilla et al., 2011). A recent study on the association of red hot chile consumption and mortality in a large American population observed a 13% reduction in mortality (Chopan et al., 2017).

Capsaicinoids are the constituents in pepper that are responsible for pungency (Sarpras et al., 2016). The degree of pungency is characterized in terms of Scoville heat units (SHU) measured based on the concentrations of capsaicinoid compounds within the fruit. SHU scale measures the number of times the extract is diluted to make pungency undetectable in sugar water (Scoville 1912). Physiologically, capsaicinoids are synthesized by the condensation of vanillyl amine produced by the phenylpropanoid pathway and a branched-chain fatty acid produced by the catabolism of amino acids. Within the placental tissues of the developing pepper fruit, capsaicinoids are synthesized 20 to 30 days after pod formation and continue to accumulate as the fruit matures (Castro-Concha et al., 2016). Some of the genes involved in the biosynthesis of capsaicinoids have been characterized in both pungent and non-pungent cultivars. Pun1 is a key regulator in the capsaicinoid pathway and controls the accumulation of capsaicinoids (Reddy et al., 2014; Stewart et al., 2007). Besides genetics, the concentration of capsaicinoids depends on other factors, such as stage of maturity and agronomic growing conditions (Islam et al., 2015; Dubey et al., 2015). Genetic diversity, agronomic practices, and environmental conditions similarly influence the accumulation of polyphenolic compounds, minerals, Vitamin A, and ascorbic acid (Sivakumar et al., 2018; Zewdie et al., 2000).

Numerous biochemical and physiological changes occur at different stages of pepper development due to changes in synthesis, transportation, and degradation of various metabolites (Menichini et al., 2009; Manikharda et al., 2018). At the mature green stage, the dominant pigments in peppers are chlorophylls and carotenoids. As maturation progresses, significant biochemical changes lead to the formation of new pigments (red/yellow carotenoids plus xanthophylls and anthocyanins). Moreover, the emission of volatile organic compounds that are associated with increased respiration, protein synthesis, formation of pectins, conversion of

chlorophylls, and changes that include taste and flavor happens at the later stages of maturation and ripening (Palma et al., 2011). Characterization of phytochemical changes in peppers that occur during maturation is essential since they could affect antioxidant activities, aroma, taste, postharvest storage, and ultimately consumer preference.

Unique growing and environmental conditions including elevated solar radiation and significant shifts in diurnal temperatures have purported benefits for the development of unique flavor attributes of fruits and vegetables (Palma et al., 2011; Harvell et al., 1997). The total acreage of peppers grown in southern Colorado is about 950 acres with a farm gate value of roughly 5.7 million dollars (personal communication Dr. Bartolo). The biochemical composition of Colorado-grown peppers, which could impart unique aroma, nutritive, and medicinal properties has never been studied. As a result, one of the primary aims of our studies was to evaluate the capsaicinoids, total phenolics, and ascorbic acid, and antioxidant activities of different *C. annuum* cultivars.

Peppers are consumed and processed in many forms. They are consumed raw in salads as well as blended into juices with other fruits and vegetables depending on consumer preferences. In addition to the fresh consumption, processors can dehydrate, pickle, cook, or roast peppers prior to consumption. Few reports have examined how heating peppers via cooking or roasting affects their phytonutrient content (Chuah et al., 2008; Ornelas-Paz et al., 2013).

In Colorado and many other states, peppers are traditionally consumed after being roasted. Our project is to investigate the changes in phytonutrients after roasting and the potential interaction with the stage of development and cultivar.

2. Materials and Methods

2.1. Chemicals

Capsaicin, dihydrocapsaicin, ascorbic acid, Folin Ciocalteu reagent, sodium carbonate, gallic acid, potassium chloride, sodium acetate, 2,2-diphenyl-1-picrylhydrazyl) reagent (DPPH), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium persulfate, trolox, quercetin, and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Pepper Cultivars

All pepper samples in this study were collected from Colorado State University's Arkansas Valley Research Center in Rocky Ford (AVRC) during the 2016 season at different pepper pod stages of green and red. Eighteen cultivars of *C. annum* and *C. chinense* species were field grown under commercial production conditions (Figure 1). Average high temperatures of Rocky Ford area in the months of July, August, and September 23.2, 25.6, 30.1 °C and minimums were 16.3, 15 and 11.2 °C respectively. Photosynthetically active radiation for those three months was 609, 482.6 and 425 W/m2, respectively. The elevation of Rocky Ford is 4180 feet above sea level. Three to five pepper pods of each cultivar were harvested from separate plants and were washed under running tap water and dried with paper towels. The peppers pods were cut into small pieces without peduncles, freeze-dried (LABCONCO, Kansas City, KS, USA), and ground to a fine powder by using a kitchen coffee grinder (Cuisinart). All samples were stored at −20 °C until further analysis.

2.3. Roasting

Five to six peppers pods were washed and dried and cut into small pieces without peduncles, placed in an oven tray, transferred to the preheated oven set at 150 °C, and roasted for

20 min in a commercial oven. The oven was preheated for uniform heat distribution. All sample pieces were cooled, freeze-dried, and then ground and stored as described above.

2.4. Extraction and Analysis of Capsaicinoid Compounds

Five hundred milligrams of ground sample from 3 to 5 pepper pods was added to a 15 mL polypropylene tube. Extraction and quantification of capsaicinoid compounds were performed essentially as described by Collins et al. (1995). Ten milliliters of methanol was added to each sample and kept in an orbital shaker overnight at 25 °C. The supernatant was transferred to a fresh 15 mL tube. Ten milliliters of methanol was added to the residue and shaken as just described. Then two supernatants were combined. One milliliter of the methanolic extract was filtered through 0.45 µm filter cartridge (Advanced Microdevices, Ambala, India) and put in a 1.8 mL sample glass vial for high-performance liquid chromatography (HPLC) analysis.

Capsaicin and dihydrocapsaicin were quantified using a Waters HPLC system equipped with a fluorescence detector and an Waters, Nova-Pak C18 4µm, 4.6 × 150 mm C18 column. Aqueous methanol A (10% methanol) and B (100% methanol) were used as eluent with a flow rate of 0.4 mL/min, and a gradient of 0 to 10 min, 80% A and 20% B. The fluorescence detector was set to an excitation wavelength of 280 nm and an emission wavelength of 338 nm. Levels of capsaicin and dihydrocapsaicin were estimated using a calibration curve with a standard of capsaicin and dihydrocapsaicin with concentrations ranging from 0.1 to 10 µg/mL. The resulting linear coefficient constants were 0.995 and 0.997, respectively.

2.5. SHU Determination

The concentration (ppm) of capsaicin and dihydrocapsaicin compounds were converted into SHU (ppm) using their coefficient of the heat value with the following formula (Scoville 1912):

$$SHU = (capsaicin \times 16.1) + (dihydrocapsaicin \times 16.1).$$

2.6. Extraction of Phenolic Compounds

Phenolic compounds were extracted from the freeze-dried material using methanol as the solvent. Five hundred milligrams of pepper powder from 3 to 5 pepper pods was mixed with 20 mL methanol in a 50 mL centrifuge tube and homogenized for 2 min followed by 2 min of vertexing. The mixture was incubated overnight at 25 °C. The supernatant was transferred to another tube, and re-extractions were done with the residue. The two supernatant liquids were combined and filtered through a 0.45 μm filter and stored at −20 °C until further analysis.

2.7. Analysis of Total Phenolics (TP) and Total Flavonoids (TF)

The TP content in the extract was determined using the Folin-Ciocalteu method, as described by Kalita et al., (2014). To measure the TP, 30 μL of the extract was mixed with 50 μL distilled water in wells of a 96-well plate. Fifty microliters of Folin Ciocalteu reagent and 80 μL Sodium carbonate (75 g/L,) was added to each well in the plate, mixed well with a pipette, and then shaken for 4 min in a plate reader. The plates were incubated for 2 h at 25 °C in the dark. The absorbance of the contents was measured with a Power wave XS2 plate reader (BioTek) at 760 nm. Gallic acid was used as the standard and TP were quantified as μg/g of gallic acid equivalent per gram freeze-dried sample.

To measure TF (30 μL) was added to 80 μL aluminum chloride (20 g/L) in a 96-well at-bottom microplate on ice. Samples were shaken for 30 sec. and then the plates were kept in the

dark at 25 °C for 1 h. The absorbance of the reaction was measured at 415 nm. Quercetin was used as the standard. TF were expressed as μg of quercetin equivalents per gram of freeze-dried weight.

2.8. Extraction and Analysis of Ascorbic Acid

Ascorbic acid was extracted from ground samples using meta-phosphoric acid and estimated using a method described by Watada et al. (1982). Five hundred milligrams of freeze-dried powder was mixed with 10 mL of 2.5% meta-phosphoric acid in a 15 mL tube. Samples were centrifuged at 5 rpm for 15 min. The supernatant was collected and filtered through 0.45 nm filter paper. The quantification of ascorbic acid was performed using a Waters 2695 HPLC system (Waters Corporation, Milford, MA, USA) equipped with a Photodiode Array Detector (PDA) and a C18 column. The flow rate was 0.4 mL/ min with gradients of A (2.5% meta-phosphoric acid, 98% methanol) and B (100% methanol). The PDA was set at an excitation wavelength of 254 nm. Ascorbic acid standards were prepared in the range of 0.1 to 10 μg/mL in meta-phosphoric acid. The concentration of ascorbic acid in unknown samples was calculated from the standard curve.

2.9. Antioxidant Activity

2.9.1. DPPH Assay

Radical scavenging activity of the extracts was evaluated using the scavenging activity of the stable DPPH free radical, which was measured as described by Kalita et al. (2013) with slight modification. In a 96-well microplate, 30 μL of sample extract was added to each well containing 20 μL of distilled water. Two hundred microliters of 60 mgL^{-1} DPPH radical solution was added and mixed thoroughly. Samples were kept in the dark for 30 min. The absorbance of this reaction was measured using an ultraviolet spectrometer at 515 nm. A control was prepared by adding 30 μL of methanol without sample extract. The DPPH radical-scavenging activity was calculated using the following formula:

DPPH radical-scavenging activity (%) = [(A control – A sample/ A control)] ×100, where A is the absorbance at 515 nm.

2.9.2. ABTS Assay

The ABTS radical cation-scavenging activity of the extracts was measured according to the method described by Kalita et al. (2013) with modifications. ABTS radical (8 mM) was prepared and mixed with (3 mM) potassium acetate and the solution was kept in the dark for 12 h. The absorbance of the solution was adjusted to 1 then 285 µL of ABTS solution was added to 15 µL sample extract in wells of 96-well plates. The absorbance of the solution was recorded at 734 nm. Trolox was used as a standard to determine the antioxidant capacity in pepper samples, and the antioxidant capacity was expressed as µmol TE/g freeze-dried sample.

2.10. Statistical Analysis

Capsaicin, dihydrocapsaicin, total phenolics, total flavonoids, and antioxidant activities of pepper samples were conducted in triplicate. The significant differences among the means were determined by analysis of variance (ANOVA) using R software version 3.4.3 for Windows. Tukey's test was performed to determine whether differences between means were significant at $P < 0.05$. All statistical analyses were performed with R software version 3.4.3 for Windows. The results were reported as mean ± standard deviation (SD) values. The Pearson's correlation test was used to assess correlations between the means.

3. Results and Discussion

3.1. Levels of Capsaicinoid Compounds

Capsaicinoid compounds are responsible for the pungency and unique taste in pepper cultivars. Twenty-three capsaicinoid analogs have been described (Sarpras et al 2016). These include capsaicin, dihydrocapsaicin, nor-dihydrocapsaicin, homo-dihydrocapsaicin, homo-capsaicin, nor-capsaicin, and nornor-capsaicin. Capsaicin and dihydrocapsaicin constitute 90% of these compounds in pepper. They are responsible for pungency and induce the sensation of hotness. Nor-dihydrocapsaicin has little effect on sensory attributes. We could detect the presence of capsaicin and dihydrocapsaicin using HPLC in select *C. annuum* and *C. chinense* cultivars. The separation and identification of these compounds are shown in Figure 2.1. We quantified the levels of capsaicin (Figure 3a) and dihydrocapsaicin (Figure 3b) in each cultivar.

In 16 pepper cultivars of *C. annuum*, the content of capsaicin ranged from 26 µg/g (Serano Mild) to 867 µg/g (CSU 243) whereas dihydrocapsaicin ranged from 13 µg/g (Serano Mild) to 489 µg /g (Mosco) in the green stage. Similarly, in the red stage capsaicin content ranged from 49 µg/g (Serano Mild) to 819 (CSU 243) whereas dihydrocapsaicin ranged from 14 µg/g (Serrano Mild) to 387 µg/g (CSU RLC) (Table 1) in 16 pepper cultivars of *C. annuum*. We could not detect capsaicin and dihydrocapsaicin in four cultivars Flavorburst, Canrio, Sweet Delilah, and Aristotle.

Habanero is a popular cultivar grown in the Rocky Ford area of southern Colorado and is known for its high Scoville heat units. *Capsicum Chinense* species including Habanero was known to be the highly pungent chili pepper (Canto-Flick et al., 2008). As expected, the highest levels of capsaicin and dihydrocapsaicin were detected in the Habanero variety (4820 and 2162.22 µg/g, respectively). The lowest levels of capsaicin and dihydrocapsaicin were found in the Serrano variety (26 and 13 µg/g respectively). There were significant differences among the cultivars of

peppers (*p-value* is set at ≤ 0.05). The findings from several studies have suggested that variations in capsaicinoid quantity can be attributed to intrinsic genetic factors of each cultivar or to the environmental conditions where they are cultivated (Sarpras et al., 2016). Islam et al. (2015) reported variation in capsaicinoid levels ranging from 0.02 to 72.05 mg/g in 139 different landraces of Capsicum. In addition to the cultivar type, accumulation of capsaicin and dihydrocapsaicin is affected by the activity of capsaicin synthase and peroxidase enzymes. The differences in the content of capsaicin are due to gene modifying factors that contribute to the accumulation of capsaicin and dihydrocapsaicin in different cultivars (Wang et al., 2006). *C. chinense* displayed the highest level of capsaicin compared to *C. frutscens* and *C. annuum*, which was correlated with the greater expression of the capsaicin regulator gene, Pun1 (Sarpras et al., 2016).

SHU is the crucial measurement to evaluate the pungency of pepper cultivars. Weiss (2002) classified the pungency of peppers into five SHU levels depending on SHU: Non-pungent (0–700 SHU), mildly pungent (700–3000 SHU), moderately pungent (3000–25,000 SHU), highly pungent (25,000–70,000), and very highly pungent (>80,000 SHU). Based on this scale in our study, most of the *C. annuum* cultivars, such as CSU 243, Fresno, CSU 256, Anaheim 118, Mosco, CSU RLC, CSU 290, Numex Joe Parker, Pueblo Chile, CSU 274, CSU 321, and Serrano mild peppers, could be grouped as moderately hot peppers. As expected, Habanero was classified as very hot. Sweet Dahlia, Flavorburst, Canario, and Aristotle were non-pungent peppers (Figure 4a, b).

Levels of capsaicinoid compounds change with maturation stage (Howard et al., 2000). The capsaicinoid and dihydrocapsacionoids levels increased at the red stage (Figure 2.3a, b). Bae et al. (2014) reported that Cayenne peppers displayed significant changes in the capsaicin and dihydrocapsaicin levels from 14.95% to 21.17%, and from 7.20% to 11.46%, respectively, at the green and red stages. A similar observation was obtained for Shimmatogarashi peppers, where the

levels of SHU increased from 46,736 to 57,995 as fruit matured to the orange and red stages (Menichini et al., 2009).

Sarpras et al. (2016) grouped 136 capsicum germplasm belonging to C. *Chinense*, C. *frutescens*, and C. *annuum* into species having 0–10000, 10000–0.1 million, 0.1–0.3 million, 0.3–0.6 million, 0.6–0.9 million, and 0.9–1.2 million SHU. The interactions between cultivars, stages and SHUs were summarized in Figure 5. The interaction plot shows that the lines of the two stages are not parallel, that means there is a statistically significant interaction between cultivars and stages except in Serrano Mild. CSU 321, CSU 290 and CSU 243 cultivars. The differences between some cultivars are not evident because they are masked by the higher values of Habanero cultivar.

3.2. Levels of Bioactive Compounds (Ascorbic Acid, TP, TF, and TA)

Ascorbic acid plays a vital role as an antioxidant compound. It is very abundant in fruits and vegetables, particularly in peppers. Ascorbic acid levels in peppers varying depending on the cultivar and agro-climatic conditions. The content of ascorbic acid of the pepper cultivars we studied ranged from 222.55 (CSU 256) to 945.36 (Fresno) mg/100 g DW in the green stage from 314.87 (Mosco) to 752.54 (CSU 256) mg/100 g DW in the red stage (Table 1.1). A wide range of ascorbic acid levels has been reported in several pepper cultivars, indicating that the differences are related to cultivar, genetics, ripening stages, and agro-climatic conditions (Howard et al., 2000; Marín et al., 2004; Zhang et al., 2004). Mozafar et al. (1994) suggested that the higher level of ascorbic acid in the matured stage was due to the light intensity and glucose level, which are the precursors of ascorbic acid.

Peppers are an excellent source of bioactive compounds including anthocyanins, and flavonoids (Antonious et al., 2006). Phenolic compounds are secondary plant metabolites that play an essential role in antioxidant activity. TP in the selected peppers ranged from 2096 to 5578 μ g/g

in the green stage with the lowest levels in Serrano and the highest levels in Habanero. In the red stage, the levels of TP ranged from 3670.50 to 7689 μ g/g with the lowest level in Pueblo chili and the highest levels in Serrano Mild.

Interestingly, Serrano accumulated the most TP in the red stage. In general, the red matured stage displayed a higher level of TP than the green stage (Menichini et al., 2009). Earlier reports suggested that ripening of fruits and vegetables is associated with the significant accumulation of TP (Belwa et al., 2019). Anthocyanins are a subgroup of orange, purple, and red colored flavonoid compounds that are present in many fruits and vegetables (Arnnok et al., 2011). Habanero 's and Flavorburst' orange-yellow-colored peppers are rich in pelargonidin (Khoo et al., 2017). Flavonoids have antioxidant activity. Presently, the TF content of the pepper cultivars ranged from 204.44 (Habanero) to 756 (Flavorburst) μ g/g in the green stage and from 557.28 (CSU 321) to 962.71 (Numex Joe E Parker) μg/g in the red stage (Table 2). TF was the lowest in the green stage of Habanero, was intermediate in Fresno (green stage), and the highest in red stage Numex Joe E. Parker. TF was the lowest in the red stage Mosco. A similar variation of flavonoids among different cultivars has been previously described (Howard et al., 2000).

3.3. Antioxidant Activities

The use of DPPH is a standard means of measuring the antioxidant capacity of fruits and vegetable extracts. The DPPH scavenging activities of pepper cultivars are shown in Table 2. Scavenging of DPPH free radicals ranged from 61% to 87% and from 59% to 87% in green and red/yellow stages of different pepper cultivars, respectively. The highest antioxidant potential in green and red stages was observed in Canrio. Antioxidant potential was the lowest in Aristotle at the green stage and Mosco at the red stage. The difference in the antioxidant activities reflected the nature and level of antioxidant compounds found in the peppers. The use of ABTS is another

way to measure antioxidant capacity. The ABTS radical cation scavenging activity of different kinds of pepper cultivars ranged from 72 to 157 µ mol Trolox/g (Table 2). Among the C. annuum cultivars, CSU 290 had the highest 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) scavenging capacity, while Fresno had the lowest in green stages.

In the matured stage, Habanero had the highest ABTS scavenging capacity and Fresno had the lowest antioxidant potential. Prior descriptions of the antioxidant activities of peppers have indicated substantial antioxidant activities in the green to red stages of all peppers using DPPH, ABTS, or oxygen radical absorbance capacity (ORAC) assays (Menichini et al., 2009; Hwang et al., 2012). However, most of the studies demonstrated that radical-scavenging activity increases as the fruit matures. Sora et al. (2015) reported that the ABTS scavenging activities by pepper seed and pulps ranged from 89.25 to 141.25 µmol TE/g for seed extracts and from 17.17 to 97.40 µmol TE/g for pulp extracts.

3.4. Correlation of TP, TF, TA, and Antioxidant Activities

Ascorbic acid, TP, and TF are the major compounds associated with antioxidant activity. Pearson correlation analysis of antioxidant activities and these bioactive compounds was carried out (Table 3). Capsaicin and dihydrocapsaicin had a positive correlation in all the pepper cultivars. However, no correlations were seen for capsaicinoids and the TP, TF, and ascorbic acid bioactive compounds. The correlation data suggested a relatively weak positive correlation of TP and TF with DPPH and ABTS antioxidant activities. TP displayed the highest correlation ($r = 0.55$) with the DPPH antioxidant assay. TF also show a positive correlation with a very poor correlation factor. These results contrasted with the previous report of very strong positive correlations of capsaicinoids, TP, and TF compounds with antioxidant activities Bae et al., (2014). Pearson correlation between the two methods of determining antioxidant activities were also low, but

positive (r = 0.17). ascorbic acid displayed a very poor positive correlation with the DPPH assay, while it was negatively correlated with the ABTS assay. Manikharda et al. (2009) reported a strong correlation between DPPH scavenging activities and TP, capsaicin, and ascorbic acid, with r = 0.9 in Shimatogarashi (*C. frutescenes*). Another study had similar findings Alvarez-Parrilla et al., (2011). However, in some cases, weak correlations were also found with the ORAC assay.

3.5. Effect of Roasting on the Levels of Capsaicin, Dihydrocapsaicin, TP, TF, and Antioxidant Activity

Cooking has a critical role in the compositional changes of peppers (Hwang et al., 2012). Due to the morphological and physiological differences among the different pepper genotypes, the changes in nutrients vary with cultivars (Gómez-García Mdel et al., 2013). Significant changes were evident in the levels of capsaicinoid compounds after roasting the peppers Table 2. In most cases, the levels of capsaicin and dihydrocapsaicin were reduced after roasting the peppers at the green stage, except for CSU RLC, Fresno, and Numex Joe E. Parker Table 1.1. A loss of capsaicinoids was evident in CSU-243, CSU-RLC, and Serrano Mild after roasting the peppers at the red stage, while the levels of capsaicinoids were reduced in CSU-243, CSU RLC, CSU-290, Pueblo chili, Mosco, and Fresno. These discrepancies in the levels of capsaicinoid compounds after cooking of cultivars might be due to their difference in the thickness of skin and physiological changes during ripening which could affect the heat permeability to the fruit materials. Previous studies on the effect of cooking on capsaicinoids also indicated contradictory results. Srinavasan et al. (1992) and Topuz et al. (2004) reported that there is a loss of 0% to 30% and 215% to 100% of the capsaicinoids in Indian Thai, and Turkish peppers after cooking.

Orneals-Paz et al. (2013) reported that moderate loss of capsaicinoids were observed after boiling in Mexican peppers, while grilling enhanced the level of capsaicinoid compounds. Heat treatment

during cooking disrupts the pepper cell wall and could affect the extractability of these compounds. There was a significant reduction in ascorbic acid after roasting in both green and red stage peppers, ranging from 8% to 80% irrespective of the stage. Similarly, loss of ascorbic acid was observed by Howard et al., (2006) in pungent peppers (Jalapeno) on heat treatment. Ascorbic acid disappears significantly after cooking due to their thermolability and solubility in water. Lawermmar et al. (2013) studied the effect of cooking on ascorbic acid content in pepper, green peas, spinach, pumpkin, and carrots. The highest loss of ascorbic acid (64.71%) was seen in peppers after 30 min. Loss of ascorbic acid in cooked peppers is due to the thermal oxidation of ascorbic acid to dehydroascorbic acid followed by hydrolysis to 2, 3 diketogluconic acid and conversion to other polymeric compounds (Gregor et al., 1996). Yadav and Shegal. (1995) reported that cooking at high temperature for a long time leads to pronounced atmospheric oxidation of food constituents. Chuah et al. (2008) described inconsistencies in ascorbic acid content after boiling of green and red peppers, suggested that the differences were due to the thickness of the pepper fruit skin. The thinner cell membrane would be more permeable to heat, which could result in the rapid leaching of ascorbic acid from the peppers. The effect of roasting on TA compounds is shown in Table 1.1.

Interestingly, in all roasted peppers the phenolic compounds increased by 1% to 106% in both green and red stages compared to fresh peppers. Since there is no biosynthesis of TP occurs after harvesting/roasting of peppers, a higher level of TP might be due to better extractability from the roasted peppers (Ornelas-Paz et al., 2010). Several studies reported that cell disruption in peppers increases the leaching of compounds into the solvents and increases the level of phenolic compounds compared to uncooked peppers. Shaimaa et al. (2016) reported that phenolic and flavonoids compounds were increased by cooking treatment of some sweet and chili pepper.

However, José de Jesús et al. (2010) suggested that the effect of cooking on peppers compounds could cause either an increase or decrease in TP. Turkmen et al. (2005) reported that boiling, steaming, and microwaving increased the phenolic content. Similar results were suggested by Orneals-Paz et al. (2013) boiling and grilling cooking enhanced the levels of TP. Contradictory reports suggested that cooking methods do not influence the phenolic content due to the inactivation of polyphenol oxidase enzyme by heat. Chuah et al. (2008) reported a significant loss of phenolic compounds from colored bell peppers during cooking. There was a reduction of antioxidant activity in cooked peppers in both the DPPH and ABTS assays, even though increased levels of antioxidants were evident after cooking due to better extractability. The antioxidant potential is a synergistic property of all the antioxidant compounds, and it depends on the nature of the compounds. After cooking, there could be a change or modification in the chemical properties of the antioxidants affecting radical-scavenging activities.

4. Conclusions

Colorado pepper cultivars are good sources of phytonutrients viz capsaicinoids, ascorbic acid, phenolic compounds, such as flavonoids. The high level of these nutrients is retained in peppers even after roasting, as is pronounced antioxidant activity. The present information will be helpful for breeders to select better parents to develop new pepper cultivars with the desired taste and pungency with health-promoting compounds.

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5. Figures and Tables

Figure 2.1. Morphological diversity of pepper pods of selected cultivars developed and grown at Arkansas Valley Research Center, Rocky Ford. All cultivars belong to *C.annuum* species except Habanero which is a *C. chinense*.

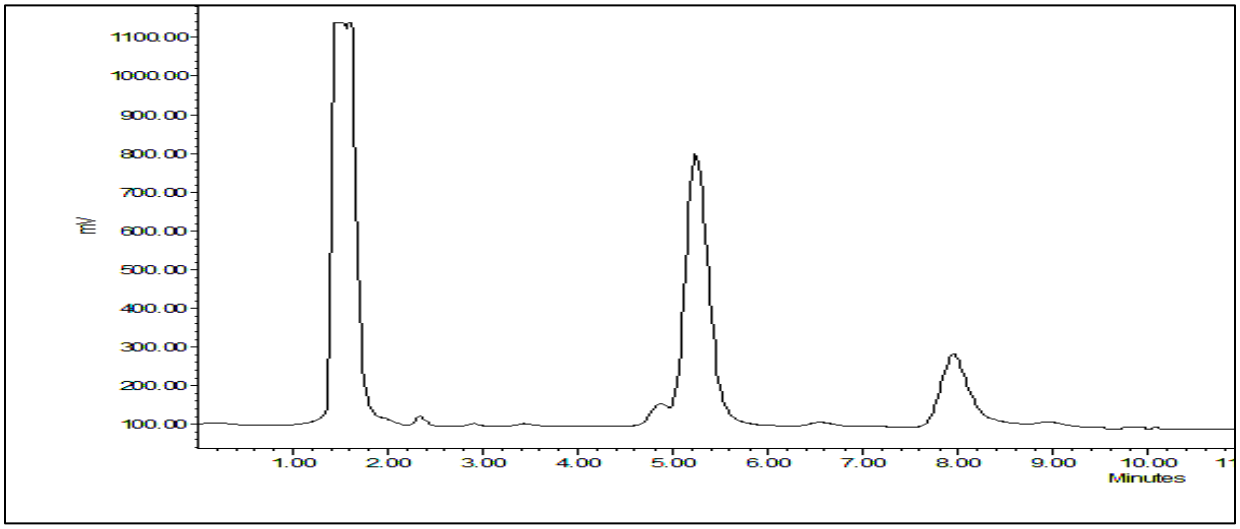

Figure 2.2 A representative high-performance liquid chromatography (HPLC) chromatogram of *Capsicum annuum* cultivar Colorado State University (CSU) 256 green showing baseline separation of (A) capsaicin, and (B) dihydrocapsaicin using Waters, Nova-Pak C18 column using a fluorescence detector. Peaks were identified by comparing retention times to those of standard compounds (capsaicin and dihydrocapsaicin).

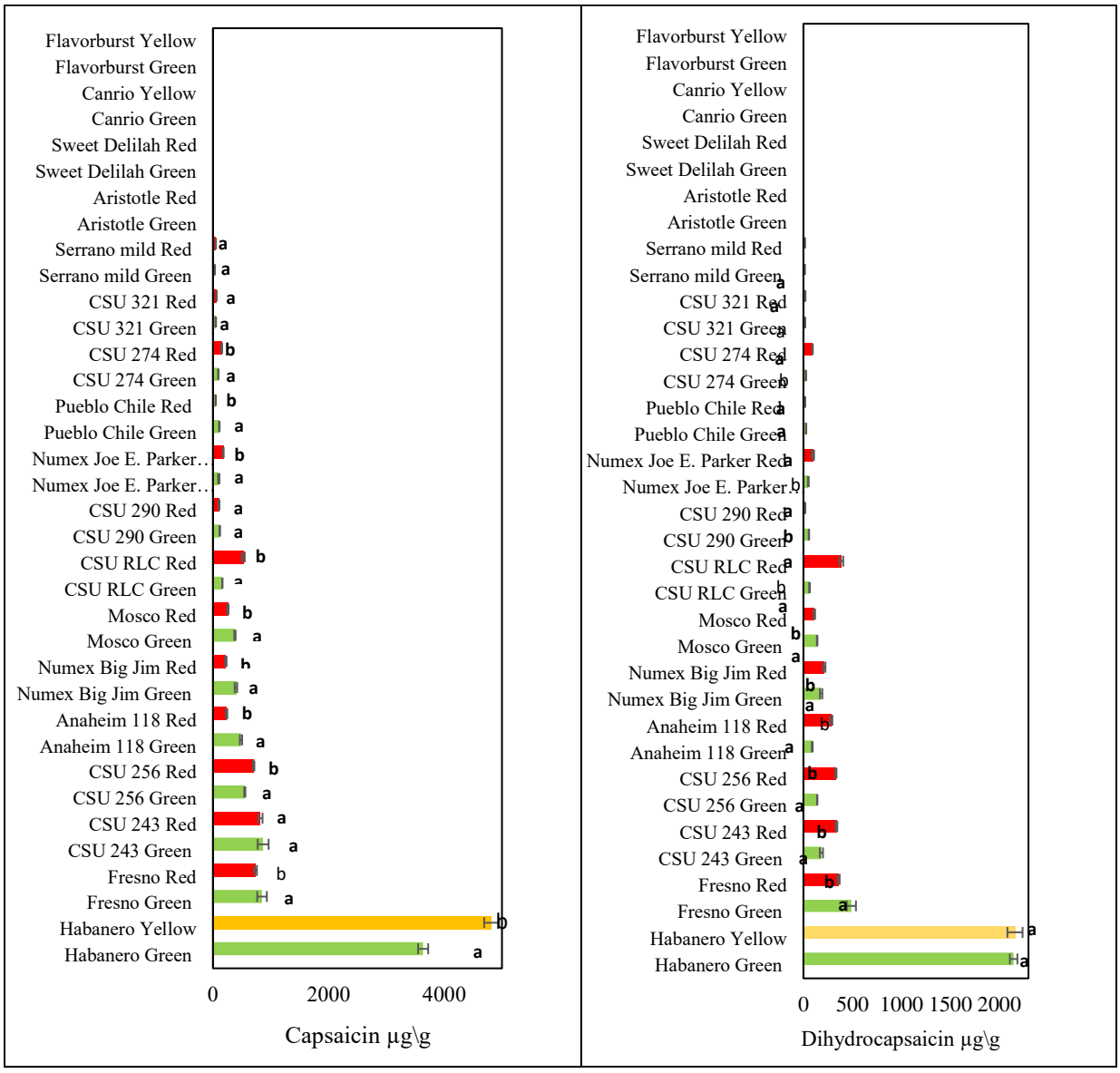

Figure 2.3 (a) Comparison of capsaicin levels in *C. annuum cultivars and Habanero.* Data are the mean of three replicates with standard deviation and are expressed as per gram freeze-dried weight. Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significant to each other. (**b**) Comparison of dihydrocapsaicin levels in *C. annuum cultivars and Habanero.* Data are the mean of three replicates with standard deviation and are expressed as per gram freeze-dried weight. Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significantly different.

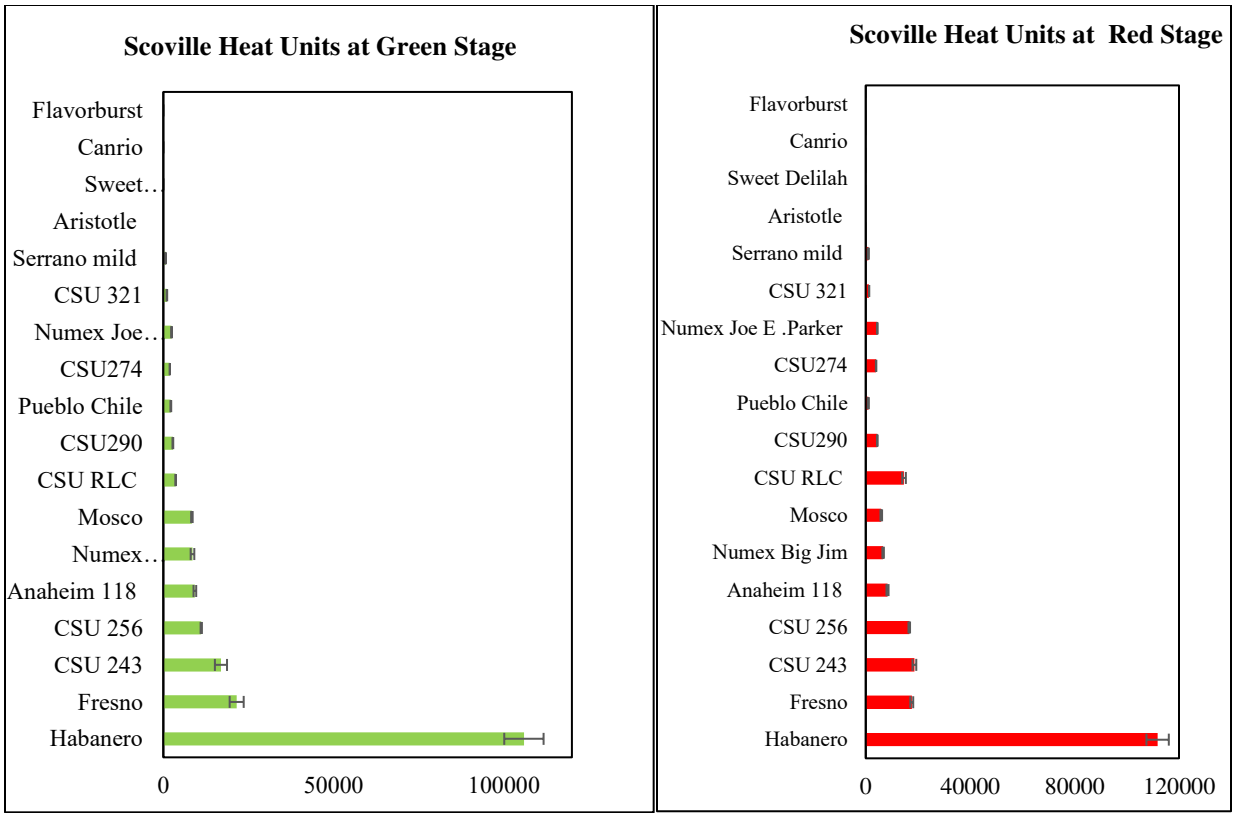

Figure 2.4 (a) Total capsaicinoids content estimated in *C. annuum* and *C. Chinense* genotypes in green pepper pods in Scoville heat units (SHU). (**b**) Total capsaicinoids content estimated in *C. annuum* and *C. Chinense* genotypes in red pepper pods in Scoville heat units (SHU). The significance level was set at $p < 0.05$. Bars represent standard deviation (SD).

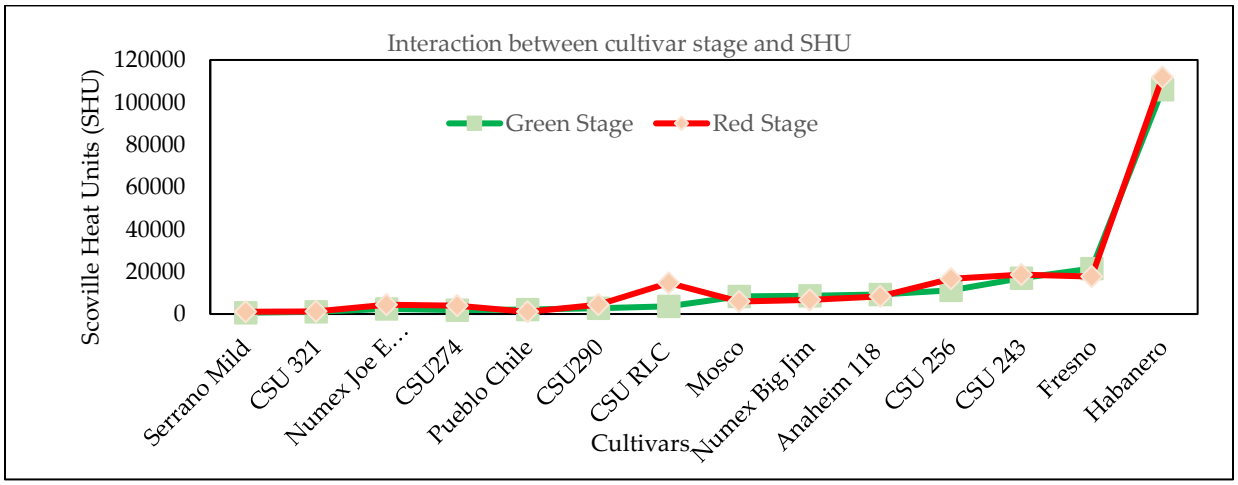

Figure 2.5. The interaction between cultivar and stage for SHU using Excel ver 2016. Cultivars that did not show capsaicin and dihydrocapsaicin were not included in this analysis.

Table 1.1. Change in content of capsaicinoid compounds in pepper pods as a function of ripening stage and cooking. Effect of roasting on the nutrient contents in *C. annuum* and *C. Chinense* viz. habanero.

Cultivars	Maturation Stage	Capsaicin (µg/g)		Dihydrocapsaicin (µg/g)		Vit. C (mg/100 g DW)		Total Phenolic (µg/g)		Total Flavonoids (µg/g)		Antioxidant Activity			
												DPPH (%)		ABTS (µg/g)	
		Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted
Flavorburst	Green	UDL	UDL	UDL	UDL	401 [a]	163↓	4279 [a]	6749↑	756 [a]	554↓	76 [a]	55↓	129 [a]	86↓
	Yellow	UDL	UDL	UDL	UDL	478 [b]	275↓	5398 [b]	6183↑	794 [b]	671↓	87 [b]	70↓	139 [a]	123↓
Canario	Green	UDL	UDL	UDL	UDL	693 [a]	328↓	5578 [a]	5783↑	500 [a]	697↑	87 [a]	61↓	110 [a]	85↓
	Yellow	UDL	UDL	UDL	UDL	1025 [b]	315↓	6316 [b]	6225↓	573 [b]	798↑	86 [a]	71↓	157 [b]	103↓
Sweet Delilah	Green	UDL	UDL	UDL	UDL	420 [a]	148↓	3314 [a]	3226↓	547 [a]	660↑	63 [a]	50↓	113 [a]	105↓
	Red	UDL	UDL	UDL	UDL	481 [b]	250↓	5115 [b]	6489↑	625 [b]	880↑	72 [b]	58↓	152 [b]	82↓
Aristotle	Green	UDL	UDL	UDL	UDL	480 [a]	180↓	2599 [a]	3252↑	329 [a]	405↑	64 [a]	54↓	107 [a]	93↓
	Red	UDL	UDL	UDL	UDL	418 [b]	217↓	4729 [b]	5318↑	556 [b]	727↑	59 [a]	41↓	156 [b]	118↓
Serrano Mild	Green	26 [a]	57↑	13 [a]	12↓	243 [a]	193↓	2096 [a]	3899↑	415 [a]	755↑	65 [a]	52↓	72 [a]	108↓
	Red	49 [a]	71↑	14 [a]	26↑	467 [b]	82↓	7689 [b]	8188↑	643 [b]	887↑	75 [b]	64↓	101 [b]	98↓
CSU 321	Green	48 [a]	19↓	16 [a]	9↓	335 [a]	175↓	2841 [a]	3277↑	443 [a]	636↑	66 [a]	62↓	106 [a]	76↓
	Red	60 [a]	61↑	19 [a]	21↑	648 [b]	405↓	5074 [b]	7117↑	557 [b]	785↑	71 [b]	55↓	126 [b]	83↓
CSU 274	Green	92 [a]	25↓	26 [a]	10↓	327 [a]	94↓	3165 [a]	3537↑	484 [a]	745↑	67 [a]	31↓	146 [a]	93↓
	Red	152 [b]	188↑	94 [b]	182↑	496 [b]	245↓	5941 [b]	7406↑	626 [b]	865↑	78 [b]	56↓	154 [a]	128↓

Pueblo Chile	Green	108 [a]	89↓	28 [a]	27↓	337 [a]	114↓	3208 [a]	4232↑	695 [a]	784↑	69 [a]	58↓	75 [a]	67↓
	Red	47 [b]	45↓	16 [a]	17↑	386 [a]	231↓	3670 [b]	5845↑	725 [a]	844↑	79 [b]	62↓	104 [b]	85↓
Numex Joe E. Parker	Green	99 [a]	225↑	50 [a]	154↑	494 [a]	105↓	3845 [a]	5958↑	656 [a]	984↑	70 [a]	65↓	111 [a]	77↓
	Red	177 [b]	251↑	97 [b]	130↑	333 [b]	243↓	4360 [b]	5019↑	962 [b]	740	81 [b]	68↓	144 [b]	125↓
CSU 290	Green	117 [a]	84↓	56 [a]	29↓	643 [a]	357↓	2769 [a]	5730↑	586 [a]	874↑	61 [a]	69↓	155 [a]	104↓
	Red	109 [a]	87↓	19 [b]	24↑	628 [a]	358↓	4448 [b]	6584↑	714 [b]	745↑	82 [b]	63↓	110 [b]	97↓
CSU RLC	Green	162 [a]	338↑	62 [a]	130↑	252 [a]	167↓	3893 [a]	5783↑	619 [a]	828↑	73 [a]	56↓	152 [a]	78↓
	Red	522 [b]	446↓	387 [b]	377↓	345 [b]	144↓	5441 [b]	7190↑	777 [b]	865↑	75 [a]	66↓	123 [b]	95↓
Mosco	Green	379 [a]	252↓	141 [a]	84↓	567 [a]	351↓	3417 [a]	4350↑	415 [a]	883↑	65 [a]	51↓	94 [a]	79↓
	Red	256 [b]	254↓	112 [b]	118↑	314 [b]	232↓	3876 [b]	4603↑	643 [b]	804↑	75 [b]	55↓	157 [b]	74↓
Numex Big Jim	Green	398 [a]	385↓	181 [a]	138↓	410 [a]	365↓	3341 [a]	4392↑	423 [a]	606↑	63 [a]	65↓	152 [a]	99↓
	Red	222 [b]	297↑	212 [b]	114↓	465 [b]	240↓	6335 [b]	7613↑	653 [b]	753↑	82 [b]	56↓	111 [b]	106↓
Anaheim 118	Green	484 [a]	454↓	90 [a]	86↓	339 [a]	211↓	3276 [a]	6367↑	504 [a]	508↑	80 [a]	68↓	121 [a]	102↓
	Red	235 [b]	325↑	282 [b]	148↓	391 [b]	254↓	4676 [b]	5580↑	641 [b]	743↑	85 [a]	69↓	150 [b]	103↓
CSU 256	Green	550 [a]	281↓	141 [a]	75↓	223 [a]	159↓	2758 [a]	2795↑	498 [a]	564↑	68 [a]	40↓	85 [a]	67↓
	Red	703 [b]	912↑	332 [b]	464↑	753 [b]	205↓	5256 [b]	6718↑	747 [b]	741	78 [b]	54↓	136 [b]	112↓
CSU 243	Green	867 [a]	512↓	183 [a]	92↓	338 [a]	79↓	4472 [a]	5538↑	634 [a]	708↑	61 [a]	49↓	120 [a]	95↓
	Red	819 [a]	514↓	337 [b]	249↓	370 [a]	214↓	5186 [b]	7950↑	704 [b]	935↑	77 [b]	60↓	145 [b]	109↓
Fresno	Green	848 [a]	729↓	489 [a]	394↓	945 [a]	464↓	3549 [a]	4624↑	713 [a]	807↑	85 [a]	72↓	159 [a]	121↓

	Red	735 [b]	599↓	359 [b]	331↓	366 [b]	167↓	4527 [b]	6444↑	792 [b]	861↑	74 [b]	67↓	137 [b]	121↓
Habanero	Green	3636 [a]	3834↑	2148 [a]	1441↓	820 [a]	160↓	4679 [a]	5041↑	204 [a]	838↑	74 [a]	59↓	153 [a]	98↓
	Yellow	4820 [b]	4876↑	2162 [a]	1572↓	349 [b]	249↓	6505 [b]	6703↑	467 [b]	627↑	86 [b]	68↓	155 [a]	122↓

Values are expressed as actual values of compounds, loss (down arrow) or gain (up arrow) from the mean of three values. Levels of ascorbic acid, total phenolics (TP), total flavonoids (TF), and antioxidant activities. Data are mean of three replicates with standard deviation and are expressed as per gram freeze-dried sample. The comparison at a specific variety is between green and red (column direction) and raw and roasted (raw direction). Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significantly different. UDL: Under detection limit. DPPH: 2,2-diphenyl-1-picrylhydrazyl). ABTS: 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid). P-value is set at ≤ 0.05

Table. 1.2. Pearson's correlation coefficient analysis among capsaicin, dihydrocapsaicin, total phenolics, total flavonoids, and antioxidant activities.

Variables	Capsaicin	Dihydrocapsaicin	Total Phenolics	Total Flavonoids	Ascorbic Acid	AA1 (DPPH)	AA2(ABTS)
Capsaicin	1						
Dihydro Capsaicin	1	1					
	<.0001						
Total Phenolics	0.4464	0.4464	1				
	0.0173	0.0173					
Total Flavonoids	-0.3906	-0.3906	0.1474	1			
	0.0399	0.0399	0.4541				
Vitamin C	0.1986	0.1986	0.2387	-0.0209	1		
	0.3109	0.3109	0.2212	0.916			
AA1 (DPPH)	0.2898	0.2898	0.5594	0.3389	0.0446	1	
	0.1347	0.1347	0.002	0.0777	0.8218		
AA2 (ABTS)	0.2981	0.2981	0.3292	-0.0695	-0.0758	0.1725	1
	0.1234	0.1234	0.0872	0.7253	0.7013	0.3801	

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CHAPTER 3: STUDYING THE INFLUENCE OF STORAGE CONDITIONS, 1-MCP, AND PACKAGING FILMS ON QUALITY OF SWEET DALILAH (*CAPSICUM ANNUUM L*)

Summary

Peppers are a popular fresh market commodity but have a limited shelf life. The present study evaluated the effects of storage time, packaging films, and 1-methylcyclopropene (1-MCP) on weight loss, firmness, respiration rate, ethylene production, ascorbic acid, antioxidant activity, and bioactive compounds of Sweet Delilah (*Capsicum annuum*). Four packaging films were tested in this study: polypropylene (P12F), laminated polynylon (30 NV), coextruded vacuum pouch (30 NVC), and polyethylene (P15G). Collectively, packaged peppers showed less weight loss than the control. When stored at the red stage, the firmness loss was 13% in peppers that were treated with 1-MCP compared to 25% loss in the control samples. The most significant reduction in respiration rate in the red stage peppers was 0.88 ml kg^{-1} h^{-1} when packaged with 30NVC and 0.91 ml kg^{-1} h^{-1} when packaged with P15G, compared to 1.22 ml kg^{-1} h^{-1} for the control. The ranges of total phenolic and total flavonoid compounds were 3782 and 5090, respectively, in the green stage and 519 and 647 µg/g, respectively, in the green and red stages. When Sweet Delilah peppers that were treated with 1-MCP maintained higher levels of phenolic and flavonoid compounds than the control samples. Overall, the largest phenolic and flavonoid losses occurred from the control samples, while the smallest phenolic and flavonoid losses occurred from the packaged peppers. The highest ABTS activity was 150 µmol TE/g when packaged with P12G film, whereas the lowest ABTS activity was 143 µmol TE/g in the control samples in the red stage. Peppers packaged with 30NVC films retained higher ascorbic acid levels than peppers that were packaged with other films and the control samples.

1. Introduction: -

Peppers are grown worldwide and have been incorporated into the cuisines of many cultures. Peppers are valued for their diverse flavors and nutritional content. In addition to their use in foods, peppers are used for coloring and valued for their medicinal properties (Ramchiary et al., 2013; Sora et al., 2015). Peppers are excellent sources of phytochemicals such as anthocyanins, vitamins, phenolic acids, flavonoids, carotenoids, and capsaicinoids (Kumar et al., 2009). These compounds act as primary antioxidants or freeradical terminators and are considered to be among the main phytochemicals that are related health benefits that include antioxidant, anti-inflammatory, and antimicrobial activities, reduced prevalence of type 2 diabetes and obesity, protection against hypercholesterolemia, and reduced prevalence of atherosclerotic cardiovascular diseases (Alvarez-Parrilla et al., 2011; Hervert-Hernández et al., 2010; Sora et al., 2015).

Peppers are often harvested at both the immature green and mature red stages. Peppers are highly perishable and require suitable postharvest handling practices to maintain food quality and decrease storage losses (Mahajan et al., 2010; Erin et al., 2018). Several factors cause pepper fruit losses after harvest, including increased respiration rate, hormone production such as ethylene, physiological disorders, and senescence (Chitravathi et al., 2016). Pepper nutritional quality is affected by the stage of fruit development, the type of processing, and the postharvest conditions during storage, transport, and handling (Chung et al., 2012). In addition, pepper nutritional quality can be affected by water loss, shriveling, tissue softening, physiological disorders, and fungal infection (Ilic et al., 2017)

There are several ways to improve pepper shelf life, such as lower storage temperature, increased humidity, and packaging with films to preserve product consistency during storage time (Sharma et al., 2013). During storage time, different treatments such as physical, chemical, and gaseous can be applied to delayed fruit ripening and preserve the high nutritional quality of peppers (Ilić et al., 2012; Mahajan et al., 2010). Studies have suggested that optimum storage temperature and high relative humidity may slow down the water loss and increase the shelf life of peppers fruits (Sharma et al., 2013). A lower storage temperature and increased humidity can extend pepper shelf life (Ilić et al., 2012). However, low temperatures may cause chilling injuries (Lim et al., 2007).

Recently, packaging films have been used to extend the shelf life and storability of perishable commodities (Manolopoulou et al., 2012). There are different types of packaging films such as Polypropylene, Laminated Poly-Nylon, Coextruded Vacuum Pouch, and polyethylene that have been used to extend the shelf life of many commodities such as vegetables and fruits. These films differ in barrier properties and selective permeability based on thickness and material. Packaging films, one of the most important techniques that have been successfully used to delay physiological processes such as water loss, respiration rate, transpiration, ethylene production, and softening and prevent decay in various vegetables and fruits (Sahoo et al., 2014; Barbosa et al., 2020). In peppers, the packaging films extend the shelf life by reducing respiration rates, chilling injury, physiological disorders (Gonzalez et al., 1999).

In addition to packaging, the 1-methylcyclopropene (1-MCP) application can have beneficial effects on fruit quality. 1-MCP is an ethylene perception inhibitor that can bind ethylene receptor molecules and delay the ripening process (Mahajan et al., 2010; Oz et al., 2011). Moreover, 1-MCP can delay ripening and senescence processes, such as pigment color changes,

cell wall softening, and processes that affect nutritional properties (Huang et al., 2003; Oz et al., 2011). Many pepper cultivars have been released in Colorado by the peppers breeding program in Arkansas Valley Research Center Colorado (AVRC), such as Joe Parker, Flavorburst, Canrio, Aristotle, and Sweet Delilah. The specialty variety Sweet Delilah is known to have a high level of bioactive compounds such as phenolics, flavonoids, and ascorbic acid but no capsaicinoid compounds (Hamed et al., 2019). It is a flavorful pepper with a short shelf life. This project's primary objective is to evaluate the effects of several packaging films and 1-MCP applications on Sweet Delilah pepper quality and to extend the shelf life to capture its true marketing potential. We measured weight loss, firmness, respiration rate, bioactive compounds, antioxidant activities, and ascorbic acid content in Sweet Delilah peppers with different packaging and storage conditions.

2.Materials and Methods

2.1. Chemicals

Ascorbic acid, Folin Ciocalteu Reagent (FCR), sodium carbonate, gallic acid, potassium chloride, sodium acetate, DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent, ABTS [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)], 1-MCP, potassium persulfate, Trolox, Quercetin, and all other reagents were purchased from Sigma Aldrich.

2.2 Pepper cultivars

The peppers varieties that were used in this study were collected from the AVRC during the 2019 season at the mature green and red stages. Pepper fruits were harvested and placed in polyethylene bags in a cold container and transferred to the San Luis Valley Research Center. For evaluation of various quality parameters, three peppers were placed in four packaging films with

different thicknesses. To estimate the content of bioactive compounds, antioxidant activity, and ascorbic acid content, three to five peppers were cut into small pieces and placed in a freeze drier until completely dry (LABCONCO New York, USA). The freeze-dried pepper samples were ground to a fine powder using a mortar and pestle and stored at −20 ° C until further analysis.

2.3 Packaging films and storage conditions

Four different packaging films with different thicknesses were tested in this study polypropylene flat bag (P12F), laminated poly-nylon (30NV), coextruded vacuum pouch (30NVC), and polyethylene (P15G) with 0.038, 0.0762, 0.038, 0.036 mm thicknesses, respectively. Three peppers were placed into their respective packaging films; then, peppers were sealed by a sealer and stored at 7.2 °C and 90% relative humidity (RH) for 21 days to estimate the quality. Unpackaged peppers (control) were stored in trays without packaging films. To simulate retail marketing conditions, peppers were kept for four days at 15.5 °C and 75% RH without packaging films. Three peppers were placed in each bag and we did three bag replications in each treatment. Three replicates of each packaging film were used in this study. A total of nine technical replicates were analyzed.

2.4 Application of 1-MCP

Pepper fruits in the red stage had been treated with 60 nl L^{-1} 1-MCP by placing the fruits in a closed container in warm water for 24 hours at 20 °C. A small fan was mounted inside the container with a battery for the gas's circulation around the fruits. Control and green stage peppers weren't treated with 1-MCP. We did not observe ethylene production in green peppers.

2.5 Weight loss

The weight loss (%) of peppers was calculated as the percentage of each sample's initial mass using (Giantex, San Diego, CA, USA) an electronic scale. The weight loss of each packaging film P15G, P12F 30NV, 30NVC, and control samples were recorded at the beginning of the experiment, 7^{th}, 14^{th}, 21^{st} and four days simulated marketing conditions.

2.6 Firmness

The Firmness of peppers in green and red stages was measured by using texture analyzer equipment (Brookfield CT3, Middleboro, MA, USA)). The firmness measurement was carried out using a cylindrical probe of 2 mm in diameter, and the speed of the probe was set to 1 mm/s. The measurement of firmness was carried out on 20 discs of peppers, and the measurement of firmness was carried out at the beginning of the experiment, 7^{th}, 14^{th}, 21^{st} and four days simulated marketing conditions.

2.7 Respiration rate and Ethylene levels

The Respiration rate and ethylene production of peppers were measured by using a Gas Analyzer (F-900 Felix Instruments Place, Camas, USA). A gas analyzer was used to measuring the level of CO_2, O_2, and C_2H_4, and the measurements of the gases were carried out at the beginning of the experiment, 7^{th}, 14^{th}, 21^{st} and four days simulated marketing conditions.

2.8. Color measurement

The color was measured with MiniScan Chromameter (Reston Virginia, USA) on CIE L*a*b* chromatic space. The instrument was initially calibrated using a white standard and black standard. Color measurements were taken on opposite sides of each pepper sample. The chroma

values of the green and red stage were calculated by using the chroma values equation. $C^* = (a^{*2}+b^{*2})^{0.5}$. C* chroma value described the intensity of color in a sample (+a*) describe the degree of red, (-a*) the degree of green while (-b*) describe blue and (+b*) describe the degree of yellow color (Manolopoulou et al., 2010)

2.9. Extraction and analysis of the phenolic and flavonoid compounds

Pepper samples weighing 0.500 g were mixed with 15 ml (80% methanol) and homogenized for 5 min. The supernatants of pepper extract were filtered to evaluate total phenolics and total flavonoids. Total phenolics and flavonoids were estimated by spectrophotometric methods, using a Costar 3370 spectrophotometer (Corning, NY, USA). The total phenolic compounds were calculated according to the method declared by Kalita and Jayanty. (2014) with modifications. Folin–Ciocalteu reagent solution was added to pepper extract, and sodium carbonate was added to the 96 microplates. The total phenolic compounds of pepper samples were calculated as gallic acid (µg/g). The colorimetric method was used for total flavonoid compounds to evaluate the total flavonoid compounds in pepper samples. Aluminum chloride was added to the pepper extract in 96 microplates. The total flavonoid compounds of pepper samples were expressed as quercetin (µg/g).

2.10 Extraction and analysis of ascorbic acid

Ascorbic acid was extracted using meta-phosphoric acid from ground samples and estimated using a method defined by Watada et al. (1982). The ascorbic acid quantification was performed using a Waters 2695 HPLC system fitted with a Photodiode Array Detector and column C18. In unknown samples, the concentration of ascorbic acid was determined from the standard curve.

2.11. Antioxidant activity

2.11. 1 DPPH assay

DPPH activity of peppers extract was estimated according to the method described by (Kalita and Jayanty, 2014). Pepper's extract (25 µl) was added to 15 µl distilled water in a 96-well microplate. Then, the DPPH solution was added to pepper extract and the absorbance of the reaction was set at 515 nm. The DPPH activity has been calculated using the following formula: DPPH activity (%) = [(A control – A sample/ A control)] ×100.

2.11.2 ABTS assay

The ABTS radical cation-scavenging activity of the pepper sample was estimated using the method described by Kalita and Jayanty (2014) with modifications. ABTS solution (280 µl) was added to 10 µl sample extract in 96 microplates. Then, the absorbance of the reaction was set at 734 nm. To evaluate pepper samples' antioxidant activity, Trolox was used as a standard, and the antioxidant capacity was expressed as µmol TE/g.

2.12 Statistical Analysis

The effects of packaging films, and storage time on weight loss, firmness, respiration rate, ethylene levels, antioxidant activity, vitamin C, and bioactive compounds were determined by analysis of variance (ANOVA) using the R software. The results were reported as mean ± standard deviation (SD) values. Tukey's test was performed to determine whether differences between means were significant at $P < 0.05$. All statistical analyses were performed with R software version 3.4.3 for Windows. The correlation analysis among the means of respiration rate and weight loss by using IBM SPSS Statistics version 28.0.0.0 (190) at $\alpha = 0.05$. Pearson’s correlation test was used to assess correlations between the means.

3. Results and discussion

3.1 Weight loss

Weight loss is one of the most important quality parameters that determine the shelf life of fruits and vegetables (Castro et al., 2002). Weight loss in fruits and vegetables during storage is primarily due to water loss, respiration, and evaporation, which depends on the temperature, relative humidity, and storage conditions (Awole et al., 2011). Our studies observed that packaging films and storage conditions affected the quality of pepper fruits during storage time (Fig. 3.1A). In the green and red stages, the weight loss in packaged peppers is less when compared to control at 7.2 °C and 90% RH for 21 days. Green peppers packaged with films lost 0.70% with (P15G), 0.72 % with (P30NVC), 0.89 with % (P30NC), and 1.06% with % (P12F) whereas control samples lost 3.37% at 7.2 °C and 90% RH for 21 days. Moreover, there was a significant difference between green pepper packaged with P15G films and P12 film compared to other packaging films.

Green peppers packaged with P15G showed the lowest loss of weight compared to other packaging. Manolopoulou et al., (2010) reported that green peppers packaged with MDPE-30 lost 0.32% of their weight, 0.65% with LDPE-60, and 1.17% with PVC unpackaged samples showed the largest weight loss (3.91%). In the case of bell peppers that were packaged with low-density polyethylene, the weight loss was less than 2% after 21 days of storage compared to that of the control samples.

Our findings exhibited that red peppers had a higher weight loss than green peppers (Fig.3.1B). Red peppers packaged with films lost 1.82% (P30NC), 2.03% (P15G), 2.35% (P30NVC), and 2.28% (P12F) whereas, control samples lost 4.91%. The rate of weight loss depends on the type of crop andthe stage of maturity of a commodity. The difference in weight loss between green and red peppers, when stored under the same temperature and humidity

conditions, may result from differences in physiological condition and respiration. These results are similar to the findings of Mahajan et al., (2016), who reported that pepper fruits were stored at 18- 20 °C with 90-95% relative humidity packaged with various films such as heat shrinkable film (15µ), cling film (15µ) and low-density polyethylene film (LDPE25µ) showed the lowest weight loss compared to control samples. There was a significant difference between red pepper treated 1-MCP stored in P30NC and peppers that were packaged with P15G films and P12 film. Furthermore, the lowest weight loss was 1.82% in packaged peppers when treated with 1-MCP, whereas the highest weight loss was 4.91% in control samples.

These results agree with data described by Ilić et al., (2012), who studied the influence of 1-MCP on postharvest storage quality of bell pepper at 7 °C and 90% RH for 18 days storage in dark conditions. They found that pepper fruits treated with 1-MCP exhibited less weight loss (3.2%) compared to control samples (3.6%). Similar results were obtained by Thakur et al., (2017), when peppers treated with 1000 ppb of 1-MCP. Our study showed that the quality attributes of peppers packaged with different films were observed to be higher than control fruits. These results suggested that pepper at red stage treated with 1-MCP showed to be the most effective treatment in maintaining fruit quality and minimizing deterioration during storage time of peppers.

3.2 Firmness

Firmness is a critical quality attribute for consumers. The use of packaging films in the food industry is essential because they provide excellent protection against changes in texture during storage time (Diane et al., 2010). Several studies have indicated that the change in firmness in fruits and vegetables is related to moisture loss through transpiration and enzymatic changes in the cell wall (Požrl et al., 2010). During storage, green pepper firmness was reduced by 3.11% with P15G, 4.35% with P12F, 4.84% in P30NVC, 4.87% with P30NV, and 12.10% in the control

peppers (Fig. 3.2A) whereas the firmness of red peppers was reduced to 13.04% with P30NVC, 16.6 % with P15G, 16.8% with P30NV, 16.9% with P12F, and 25.07% in control peppers (Fig. 3.2B). Tsegay et al. (2013), reported that the firmness of sweet bell pepper decreased with an increase in storage time. The results demonstrated that red peppers that were treated with 1-MCP and stored with P30NVC films maintained a higher level of firmness than peppers that were packaged with other packaging films and the control samples Thakur et al. (2017) found that pepper fruits that were treated with 1-MCP (1000 ppb) exhibited the highest mean texture compared to control fruits. Grzegorzewska et al., (2020) reported the treatment of pepper fruits with various concentrations of 1-MCP (1.0 µl·dm-3 , 3.0 µl·dm-3 , and 5.0 µl·dm-3) at 0 °C and 5 °C for up to 8 days slowed softening compared to untreated fruits. In our study, peppers treated with 1-MCP maintained a high level of firmness throughout 21 days at 7.2 °C and four days in retail marketing conditions.

3.3 Respiration rate and ethylene levels

Respiration is one of the most important physiological processes that occur in all commodities. Decreasing the respiration rate is one of the primary objectives after harvest to delay the ripening process and preserve the quality of vegetables and fruits. We estimated the levels of respiration rate in green peppers and red peppers during storage time and compared them with control samples (Fig 3.3). In green peppers, the highest respiration rate was in control samples, whereas the lowest rate of respiration rate was in packaged peppers with (P12F) films. It was observed that the respiration rates in packaged peppers with films decreased with increasing storage time in both the green and red stages compared to those of the control samples. These results are in agreement with those of Hameed et al. (2013), who found that pepper fruits that were

stored at 10 °C and 90-95 ±RH exhibited the lowest respiration rate compared to control samples. Similar observations were made by Manolopoulou et al. (2012), who observed that the levels of O_2 were decreased in packaged peppers whereas the levels of CO_2 were increased during storage. Singh et al., (2014), observed that storage temperatures influenced respiration rates, and the smallest decrease in respiration rate was measured at 10 °C during the storage period. Pearson correlation analysis of respiration rate and weight loss and was carried out (Table 2.4). The respiration rate and weight loss had a negative correlation in all packaging films in both green and red stages respectively.

A significant difference was observed between red peppers treated with 1-MCP and packaged with P30NVC films and untreated red peppers that were packaged with P12 and 15G films after three weeks of storage. The largest decrease in respiration rate was 0.88 ml kg^{-1} h^{-1} in peppers that were treated with 1-MCP, whereas the lowest respiration rate was 1.02 ml kg^{-1} h^{-1} in the control samples. Thakur et al. (2017) reported that the lowest mean respiration rate was observed in pepper fruits that were treated with 1000 ppb 1- MCP at 10 °C and 90-95% RH for 28 days of storage compared to control samples. Studies have demonstrated that ethylene exhibits both beneficial and deleterious effects on produce. Shorter storage life, promotion of senescence, fruit softening, and discoloration are examples of deleterious effects of ethylene on peppers and other vegetables and fruits (Mahajan et al., 2016).

The application of 1- MCP has been shown to delay the ripening process by slowing the respiration rate in several vegetables and fruits. Significant variations in ethylene production level were observed in peppers that were packaged and peppers that were treated with 1-MCP compared to the level of the control (Fig 3.4). We observed that ethylene production was decreased in all peppers that were packaged and peppers that were treated with 1-MCP during storage compared

to that of the control. Our data show that the lowest ethylene production level was 9.25 ppm kg^{-1} h^{-1} , measured in peppers treated with 1-MCP, whereas the highest ethylene production was 13.6 ppm kg^{-1} h^{-1} , which was measured in the control samples. Fernández-Trujillo et al. (2009) reported that red pepper treated with 900 ppb of 1-MCP and storage at 8 °C in polypropylene package inhibited ethylene production. Huang et al. (2003) found that pepper fruits that were treated with 250 nmol/liter of 1-MCP and stored at 20 °C in polyethylene bags (0.02-mm thick) for 18 days showed less ethylene production than untreated fruits.

3.4 Color change

The change of color is an important indicator of the maturity and quality of fresh pepper. The color intensity in pepper fruits is one of the essential quality parameters that determine acceptance (Alcock and Bertling., 2013). Due to differences in carotenoid composition, peppers exhibit a range of colors, including green, yellow, orange, and red. Several studies have suggested that variations in color change can be attributed to physicochemical reactions, such as biochemical synthesis and metabolic interconversion of xanthophylls and carotenoids associated with the ripening process (Marcus et al., 1999). Therefore, we estimated the changes in color in packaged peppers with various films and peppers at the red stage that were treated with 1-MCP during the storage time.

During storage time, chroma values of fruits and vegetables can change rapidly with storage conditions. At the end of storage time, the chroma values of green peppers were 20.19 with P30NVC 20.31 with P12F, 20.52 with P30NV, 20.92 withP15G, and 19.62 in control peppers (Fig. 3.5A). The chroma values at red stage peppers showed a significant difference during the storage time with control peppers (Fig. 3.5B). After two weeks of storage, there was a significant difference between green pepper packaged with P30NVC and P15G films. Overall, we observed

that the chroma values of the green peppers decreased, whereas the chroma values of the red peppers increased. We found that the peppers that were packaged with films maintained their green color better than the controls. Significant differences were observed between red peppers treated with 1-MCP and stored in P30NVC and untreated peppers packaged with P12F. Lim et al., (2007) and GonzalezAguilar et al. (1999) reported that the chroma values of green peppers that were stored in low-density polyethylene maintained green color even after four weeks. The red peppers samples that were treated with 1- MCP showed a significant delay in the ripening processes and color change during storage. According to a study that was conducted by Fox et al. (2005), the chroma values increased from 24.2 to 29.9 in bell peppers that were stored at 20 °C with 90% RH for ten days. The chroma values increased with increasing RH during storage at 15 °C according to the study conducted by (Nunes et al., 2012). FernándezTrujillo et al. (2009) found that when peppers were treated with 900 ppb1-MCP and stored at 8 °C in polypropylene bags showed an increase in chroma values during storage. Various studies have suggested that 1-MCP treatment delays the color change of pepper fruits by inhibiting ethylene production during storage.

3.5 Phenolic and flavonoids and ascorbic acid

Peppers are an excellent source of bioactive compounds, including carotenoids, phenolics, and flavonoids. The total phenolic (TF) and total flavonoid (TF) contents of the green and red stages are presented in Table 2.2. At the beginning of the experiment, there were no significant differences in TP level among peppers that were packaged with various films and control samples in either stage. Substantial variations ($p \leq 0.05$) in TP content were observed in green peppers that were packaged with various films. In the green stage, the smallest TP loss was 4.93%, which was observed in peppers that were packaged with P30NV, whereas the largest TP loss was 11.98%,

which was observed in the control samples. The results demonstrated that the TP levels decreased with increasing storage time, and the largest TP loss was established in the control samples. Red pepper fruits that were treated with 1-MCP in P30NVC films showed the smallest loss of phenolic compounds, whereas the control showed the largest loss of phenolic compounds. The TF levels in green peppers that were packaged with the considered films followed the order P15G > P12F > P30NV > P30NVC > control samples.

There was a significant loss in TF during storage; the largest loss of TF was 7.77% in the control samples, whereas the smallest loss of TF was 3.79% in P30NV. The decrease in phenolic compounds is attributed to oxidation by polyphenol oxidase (Yamaguchi et al., 2003). Szwejda-Grzybowska et al. (2016) reported that pepper fruits that were stored at 5 °C for four days exhibited decreases in polyphenol content by 2– 7% in the Blondy variety and 11–20% in the Yecla variety. Many researchers reported similar results that TP in peppers decreases with increasing storage time (Barbagallo et al., 2012; Haishan et al., 2019; Iqbal et al., 2015). Chung et al. (2012) reported that peppers treated with 90 ppb1-MCP and stored at 10° C in polyethylene bags (50 μm) maintained high levels of phenolic content. The results demonstrated that peppers that were packaged with various films and treated with 1-MCP showed increased stability of bioactive compounds during storage. Additionally, the packaging of peppers with various films effectively slowed the decreases in the total phenolic and total flavonoid contents in both the green and red stages.

Vitamin C is one of the most important bioactive compounds in peppers which plays an essential role as an antioxidant compound (Zhang et al., 2003). A wide range of ascorbic acid concentrations have been reported in pepper cultivars; hence, the differences are related to the type of variety, genetic variation, ripening stage, and climatic conditions (Kumar et al., 2009). Several

studies suggest that the level of ascorbic acid in fruits is associated with carbohydrate metabolism. The level of ascorbic acid is high in ripening fruits due to the accumulation of sugars during the advanced ripping process (Campos et al., 2013)

A significant variation ($p \leq 0.05$) was observed in ascorbic acid content between the packaged peppers and controls (Table 2.2). The results demonstrated that the red peppers had the highest level of ascorbic acid content at the beginning of storage time, whereas the green peppers had the lowest level. During storage, the largest loss of ascorbic acid was observed in the control samples, whereas the smallest loss of ascorbic acid was in peppers that were packaged with P30NVC film. Our data showed that the ascorbic acid level decreased as the storage time was increased in the packaged peppers and control samples. During the red stage storage period, the largest loss of ascorbic acid was 4.9% in the control samples, whereas the smallest loss of ascorbic acid was 3.2% in peppers that were packaged with P12G films. Various studies reported similar results on the loss of ascorbic acid in peppers during storage (Sahoo et al., 2014; Haishan et al., 2019; Chávez-Mendoza et al., 2015)

3.6 Antioxidant activity

Antioxidant compounds of fruits and vegetables play an essential role in reducing the risk of several chronic diseases (Singh et al., 2015). Antioxidant activity is associated with bioactive compounds such as vitamin C, phenolic, and flavonoid compounds. Antioxidant compounds can inhibit free radical compounds due to the redox properties of their hydroxyl groups (Palma et al.,

2015). The difference in antioxidant activity between green and red peppers could be explained by their differences in carotenoid, phenolic, and flavonoid contents (Sun et al., 2007).

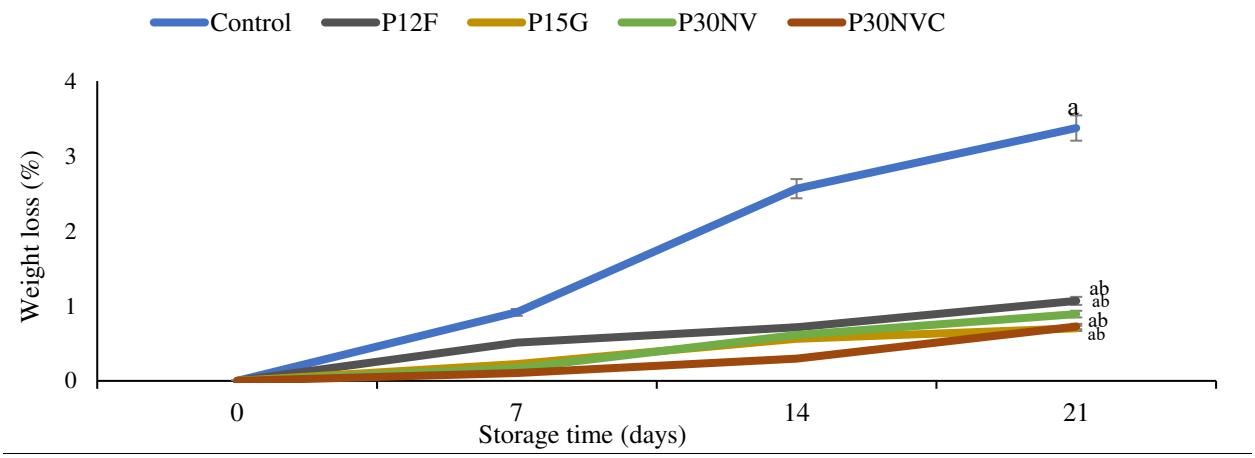
The present study evaluated the antioxidant activities of green and red peppers that were packaged with films and those of control samples. The results of this study demonstrated that packaged peppers and control samples in the green stage did not show significant differences in DPPH or ABTS activity. Kevers et al. (2007) reported that green pepper storage does not negatively affect antioxidant capacity. Our results demonstrated that red peppers had higher DPPH activity than green peppers ($p \leq 0.05$). This is due to the presence of more total phenolics and ascorbic acid in the red stage than in the green stage (Table 2.3). During storage, the largest loss of ABTS activity was 5.86% in the control samples, whereas the smallest loss of ABTS activity was 1.9% in the red peppers that were packaged with P15 films. The reduction in antioxidant activity could be related to the loss of antioxidant compounds, such as total phenol and Lascorbic acid, during storage (Haishan et al., 2019). We found that the antioxidant activity decreased with advanced storage time in both the green and red stages, which agrees with the findings of (Chitravathi et al., 2015; Devgan et al., 2019). According to this study, pepper fruits packaged with various films and pepper fruits treated with 1-MCP showed higher antioxidant activity during storage.

4. Conclusions

Sweet Delilah pepper (*Capsicum annuum L*) in both green and red stages packaged with different films were tested during storage time and marketing conditions. Packaged peppers with P15G and P12F films presented significantly less weight loss, lower ethylene and respiration rates, and less texture change compared to the control peppers. Also, the results indicate that using 1-

MCP can effectively delay weight loss, color change, maintain firmness, extend the shelf life and retain the nutritional value of peppers. Finally, packaged Sweet Delilah peppers treated with 1-MCP effectively slowed down the loss of total phenolic, total flavonoids, and ascorbic acid in the red stage. The data suggest that packaged peppers preserved more phenolics and retained antioxidant activity compared to control samples.

5. Figures and Tables



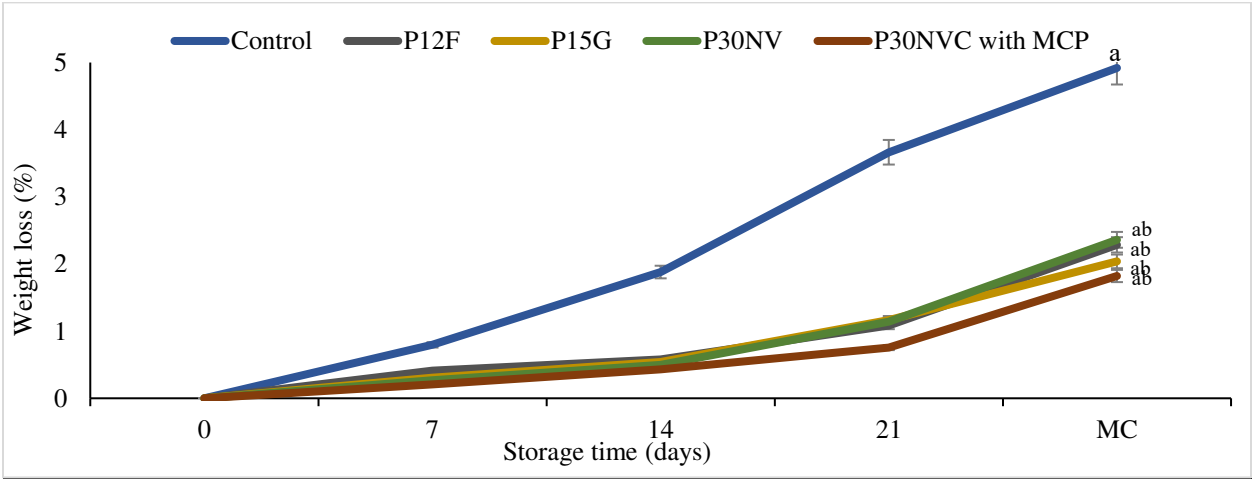


Figure 3.1. Percent weight loss in Sweet Delilah at green stage (A) red stage (B) at 7.2 °C and 90% humidity. Data are the mean of six replicates with standard deviation. Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significantly different. MC: marketing conditions (after 4 days).

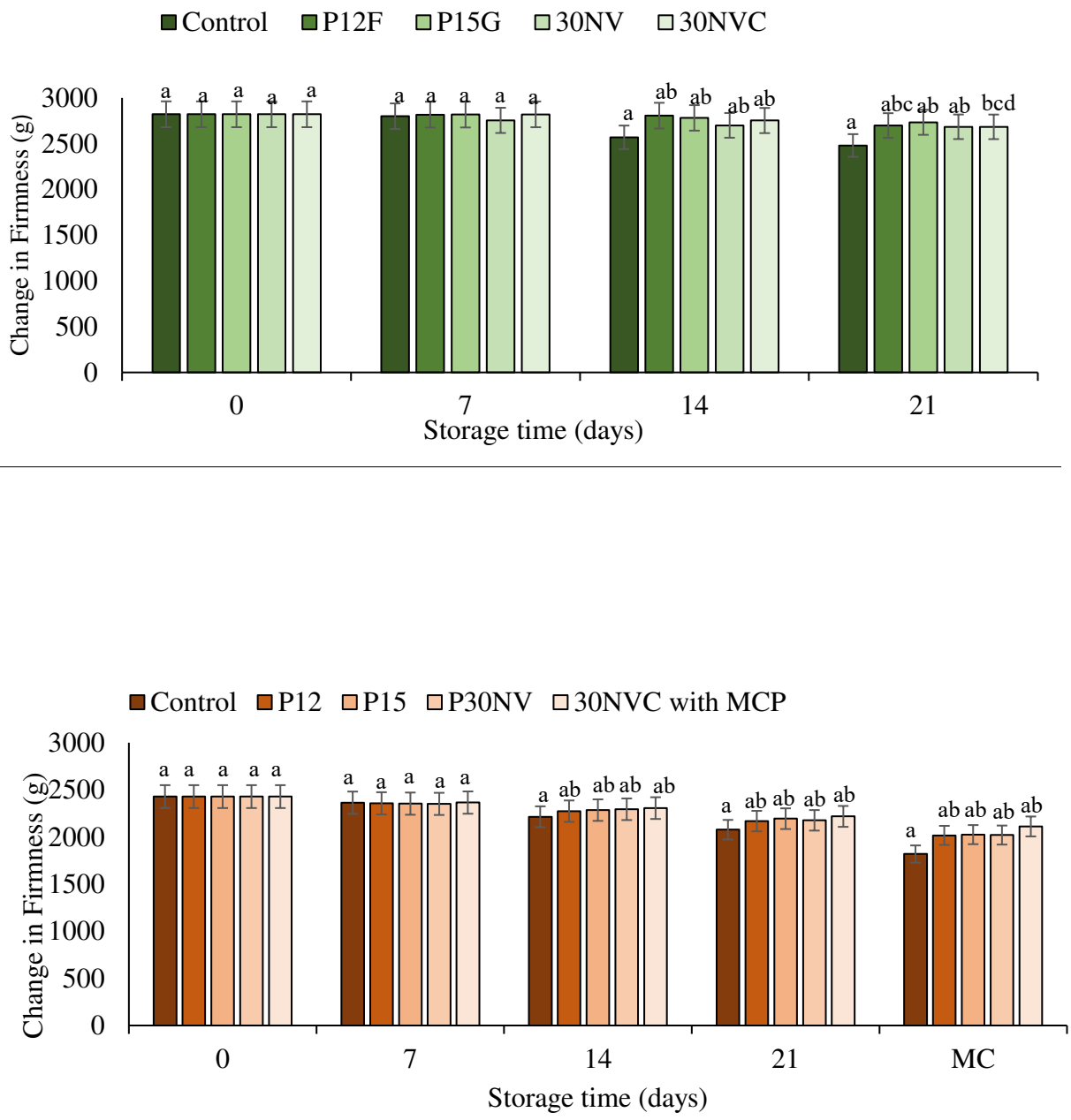


Figure 3.2. Firmness levels in green stage (A) and red stage (B) of Sweet Delilah at 7.2 °C and 90% humidity. Data are the mean of six replicates with standard deviation. Significant differences are denoted by different letters, while the same letters indicate that they are not significantly different. MC: marketing conditions (after 4 days).

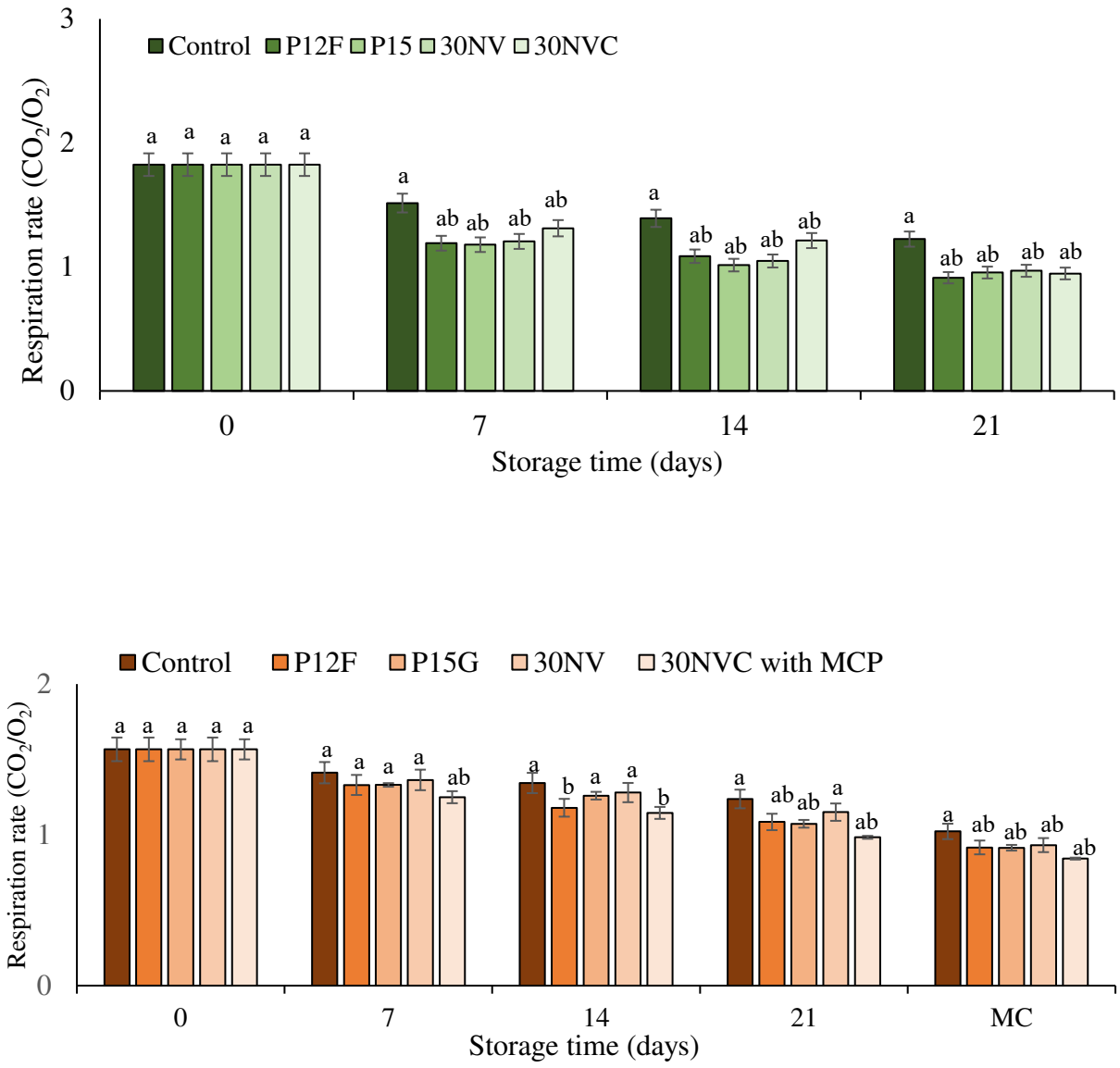


Figure 3.3. Respiration rate in Sweet Delilah green stage (A) and red stage (B) at 7.2 °C and 90% humidity. Data are the mean of six replicates with standard deviation. Significant differences are denoted by different letters, while the same letters indicate that they are not significantly different. MC: marketing conditions (After 4 days).

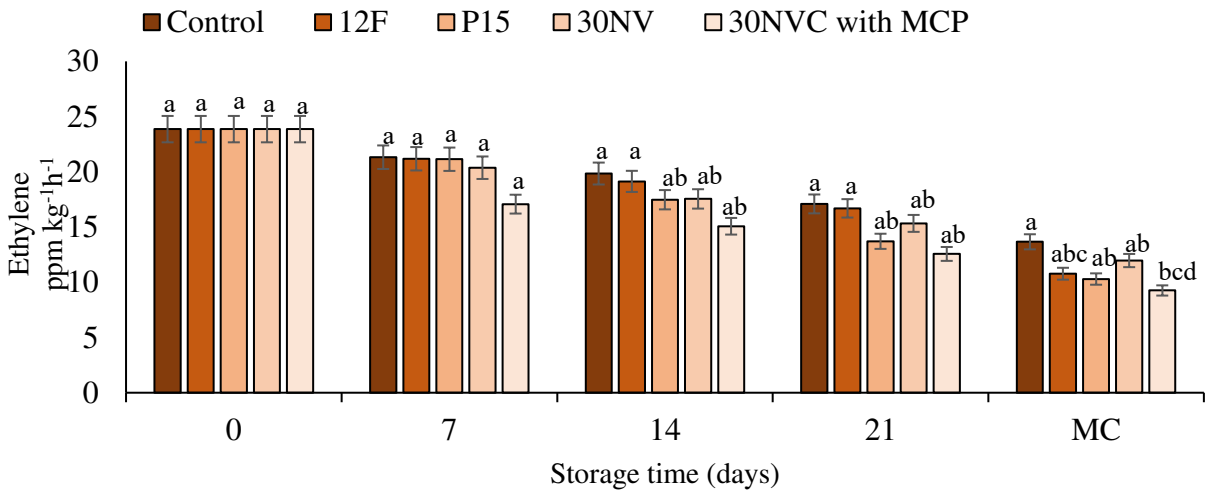
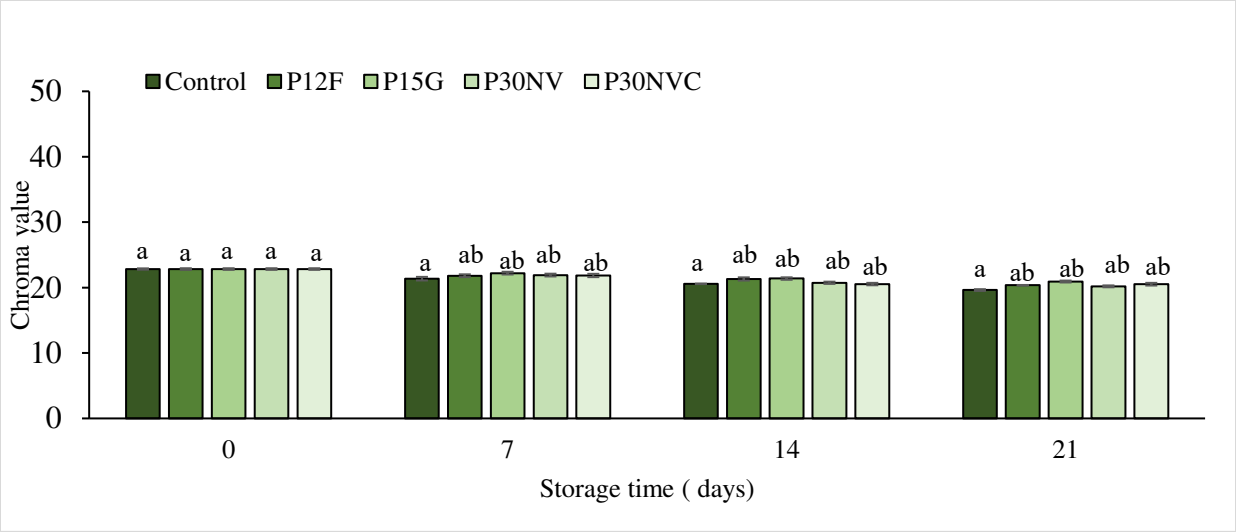


Figure 3.4. Ethylene levels in Sweet Delilah at red stage at 7.2 °C and 90% humidity. Data are the mean of six replicates with standard deviation. Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significantly different. MC: marketing conditions (After 4 days).



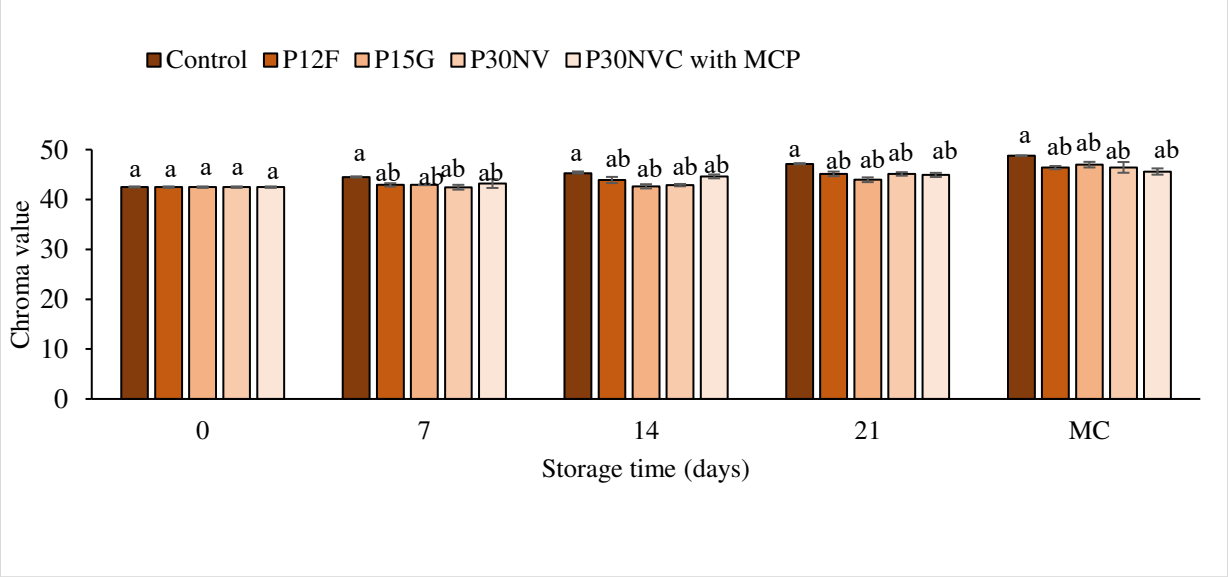


Figure 3.5. Chroma values in Sweet Delilah at green stage (A) and red stage (B) at 7.2 °C and 90% humidity. Data are the mean of six replicates with standard deviation. Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significant. MC: marketing conditions (After 4 days)

Table 2.1. The relative value of permeabilities for the most commercial packaging.

Type of package	Thickness (mm)	Permeabilities		Density, g/cm ^{3}
		O_2	CO_2	
Polypropylene (P12F)	0.0380	4.3	13.6	0.87
Laminated Poly-Nylon (30NV)	0.0762	3.4	18.4	0.90
Coextruded Pouch (30NVC)	0.0381	3.1	10.7	0.89
Polyethylene (P15G)	0.0364	3.2	11.9	0.88

Table 2.2. Change in content of phenolic, flavonoid, and ascorbic acid content in green and red stages of Sweet Delilah at 7.2 °C and 90% humidity. Values of total phenolic and total flavonoids are expressed as (µg\g). Data are mean of six replicates with standard deviation. Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significantly different. NM: not measured. MC: marketing conditions (after 4 days).

Bioactive compounds

		Total phenolic (µg/g)					**Total flavonoids (µg/g)**					**Ascorbic acid (mg/100g)**				
Stages	Packaging films	Zero Time	7th	14th	21st	MC	Zero Time	7th	14th	21st	MC	Zero Time	7th	14th	21st	MC
	P15G	3785^{a}±116	3715^{a}±138	3693^{a}±20	3576^{b}±18	NM	520^{a}±31	517^{a}±35	515^{a}±16	505.16^{a}±42	NM	420^{a}±25	418.4^{a}±8	415^{a}±21	415^{a}±13	NM
	P12F	3785^{a}±116	3706^{a}±120	3697^{a}±36	3581^{b}±28	NM	520^{a}±31	514.3^{a}±39	509^{a}±11	504.46^{a}±31	NM	420^{a}±25	415^{a}±8	413^{a}±9	411^{a}±6	NM
Green	P30NVC	3785^{a}±116	3717^{a}±29	3684^{a}±61	3568^{b}±16	NM	520^{a}±31	518^{a}±16	512^{a}±4	503^{a}±8	NM	420^{a}±25	417.4^{a}±3	415^{a}±25	405^{a}±13	NM
	P30NV	3785^{a}±116	3726^{a}±155	3655a±64	3598b±67	NM	520^{a}±31	515^{a}±13	511^{a}±49	502^{a}±20	NM	420^{a}±25	417^{a}±6	416^{a}±4	421^{a}±3	NM
	Control	3785^{a}±116	3694^{a}±35	3548^{a}±26	3331^{a}±127	NM	520^{a}±31	510^{a}±41	501.4^{a}±4	490^{ab}±19	NM	420^{a}±25	412^{a}±9	389.2^{a}±45	371^{ab}±3	NM
	P15G	5090^{a}±242	5082^{a}±279	4994^{a}±29	4882^{a}±34	4778^{a}±47	647^{a}±37	639^{a}±31	633.2^{a}±42	628^{a}±52	622.3^{a}±48	497^{a}±9	491^{a}±2	488^{a}±3	482^{a}±3	480^{a}±3
	P12F	5090^{a}±242	5067^{a}±203	4915^{a}±82	4850^{a}±80	4807^{a}±30	647^{a}±37	642^{a}±29	634.4^{a}±36	625^{a}±37	614^{a}±41	497^{a}±9	493^{a}±4	485.1^{a}±1	483^{a}±6	480.4^{a}±1
Red	P30NVC + MCP	5090^{a}±242	5035^{a}±267	5026^{a}±85	4912^{a}±61	4882^{a}±52	647^{a}±37	642^{a}±45	634^{a}±38	622^{a}±33	615.11^{a}±6	497^{a}±9	489.4^{a}±2	485.3^{a}±1	482^{a}±7	478^{a}±4
	P30NV	5090^{a}±242	5047^{a}±257	5007^{a}±290	4791^{a}±133	4773^{a}±75	647^{a}±37	639^{a}±33	636^{a}±35	624^{a}±37	610.4^{a}±19	497^{a}±9	491.2^{a}±2	486.1^{a}±1	484^{a}±2	479^{a}±1
	Control	5090^{a}±242	4900^{a}±230	4773^{a}±190	4406^{ab}±107	4304^{ab}±24	647^{a}±37	633^{a}±43	627±^{a}51	620^{ab}±46	597^{ab}±9	497^{a}±9	487^{a}±2	479.3^{a}±1	475^{ab}±0.9	472.3^{ab}±0.3

Table 2.3. Change of antioxidant activity in green and red stages of Sweet Delilah at 7.2 °C and 90% humidity. Values of DPPH are expressed as percentage, and ABTS are expresses as (µmol TE/g). Data are mean of six replicates with standard deviation. Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significantly different. NM: not measured. MC: marketing conditions (after 4 days).

	Antioxidant Activity										
		DPPH (%)					**ABTS (µmol TE/g)**				
Stages	Packaging films	Zero time	7th	14th	21st	MC	Zero time	7th	14th	21st	MC
	P15G	68a±11	68a±11	67a±10	65a±11	NM	122a±5	119a±8	115a±8	114a±1	NM
	P12F	68a±11	67a±11	67a±12	65a±12	NM	122a±5	118a±1	115a±4	114a±1	NM
Green	P30NVC	68a±11	67a±11	67a±11	65a±12	NM	122a±5	118a±2	116a±1	113a±2	NM
	P30NV	68a±11	68a±11	66a±11	66a±11	NM	122a±5	118a±1	115a±1	112a±3	NM
	Control	68a±11	65a±11	64a±12	61a±13	NM	122a±5	114a±1	113a±5	108a±7	NM
	P15G	72a±11	71a±3	70a±4	66a±8	65a±6	156a±2	154a±1	153a±1	153a±1	148a±4
	P12F	72a±3.	70a±4	69a±3	67a±2	66a±6	156a±2	154a±1	152a±2	151a±1	150a±1
Red	P30NVC + MCP	72a±3	71a±4	71a±4	67a±4	66a±1	156a±2	154a±1	151a±1	150a±0.2	149a±2
	P30NV	72a±3	70a±2	69a±3	68a±1	65a±1	156a±2	153a±2	152a±2	149a±2	148a±3
	Control	72a±3	70a±7	67a±5	64a±1	54ab±1	156a±2	153a±1	148a±3	146ab±2	143ab±3

Table 2.4. Pearson's correlation coefficient analysis between respiration rate and weight loss in at green stage and red stages of Sweet Delilah packaged with different films.

Variables	**Green stage**			
	Respiration Rate			
Weight loss	P12F	P15G	30NV	30NVC
P12	1			
	0.016			
	-0.99			
P15G		1		
		0.061		
		-0.95		
30NV			1	
			0.023	
			-0.792	
30NVC				1
				0.025
				-0.99
	Red stage			
	P12F	P15G	30NV	30NVC+MCP
P12	1			
	0.065			
	-0.95			
P15G		1		
		0.004		
		-.091		
30NV			1	
			0.094	
			-0.989	
30NVC+MCP				1
				0.150
				-0.972

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CHAPTER 4: THE BIOACCESSIBILITY OF PHENOLICS, FLAVONOIDS, CAROTENOIDS, AND CAPSAICINOID COMPOUNDS: A COMPARATIVE STUDY OF COOKED POTATO CULTIVARS MIXED WITH ROASTED PEPPERS VARIETIES.

Summary

An in vitro method was used to assess the bioaccessibility of phenolics, flavonoids, carotenoids, and capsaicinoid compounds in different cooked potatoes mixed with roasted peppers (Capsicum annuum), Joe Parker (JP, hot), and Sweet Delilah (SD, sweet). The present study identified differences in the bioaccessibility of bioactive compounds among the potato cultivars (Solanum tuberosum) Purple Majesty (PM; purple flesh), Yukon Gold (YG; yellow flesh), Rio Grande Russet (RG; white flesh) and a numbered selection (CO 97226-2R/R (R/R; red flesh)). The bioactive compounds and capsaicinoid compounds in potatoes and peppers were estimated before and after in vitro digestion. Before digestion, the total phenolic content of potato cultivars mixed with JP was in the following order: R/R > PM > YG > RG. The highest levels of carotenoids were 194.34 µg/g in YG and 42.92 µg/g in the RG cultivar when mixed with roasted JP.

The results indicate that the amount of bioaccessible phenolics ranged from 485 to 252 µg/g in potato cultivars mixed with roasted JP. The bioaccessibility of flavonoids ranged from 185.1 to 59.25 µg/g. The results indicate that the YG cultivar mixed with JP and SD showed the highest phenolic and carotenoid bioaccessibility. In contrast, the PM mixed with JP and SD contained the lowest phenolic and carotenoid bioaccessibility. Our results indicate that the highest flavonoid bioaccessibility occurred in R/R mixed with roasted JP and SD. The lowest flavonoids bioaccessibility occurred in PM and the RG. The maximum bioaccessible amount of capsaicin was observed in YG mixed with JP, while the minimum bioaccessibility was observed with PM.

1. Introduction

Crops such as potatoes (*Solanum tuberosum L.*) and peppers (*Capsicum annuum L.*) are the most common food crops in human daily diets; they are members of the Solanaceae family (Gebhardt 2016; Kumari et al., 2017). These crops have been reported as an excellent source of various phytochemical compounds such as vitamins, phenolics flavonoids, carotenoids, anthocyanins, and capsaicinoid compounds (Kumar et al., 2009; Howard et al., 2000). Due to the high abundance of bioactive compounds, these crops exhibit many nutritional and health benefits when consumed at appropriate levels (Spillar et al., 2008; Careaga et al., 2003; Alvarez et al., 2011; Vulic´ et al., 2019), making these foods highly desirable choices for frequent consumption. The biological effects of bioactive compounds such as antioxidant activity, antimicrobial, and anticancer depend on their bioaccessibility (Ifie et al., 2018). These compounds vary widely in their chemical structures and biological function; thier bioaccessibility is not well understood (Shahidi et al., 2019). The bioactive compound's concentration and stability during food processing depend on several factors: the type and cultivar, growing conditions, geographical location, postharvest handling, processing conditions, and cooking methods (Leong and Oey., 2012; Pugliese et al., 2014).

It is important to obtain information about their bioaccessibility from the foods matrix and the factors that determine their bioaccessibility (Rayan et al., 2008). The bioaccessibility of bioactive compounds describes as the part of the compound that is released from the food matrix and becomes available for absorption (Buggenhout et al., 2010; Tagliazucchi et al., 2010; Pugliese et al., 2013; Cillaa et al., 2018; Thakur et al., 2020). The combination of various food ingredients and cooking methods may significantly influence the bioaccessibility of bioactive compounds.

Potatoes and peppers are some of the most frequently consumed crops in the world. Several studies have reported that it is possible to estimate the bioaccessibility of bioactive compounds by evaluating the quantity transferred to the micelle fraction following a simulated in vitro digestion procedure (Thakur et al., 2020). Several methodologies may be used to assess bioactive compounds' bioaccessibility; among them, the most common is in vitro digestion (Veda et al., 2006). The bioaccessibility of bioactive compounds depends on various factors, which include abundance of bioactive compounds within the food matrix, heat processing, and food additives such as dietary fat, oil, fat, and certain enzymes. (Leong and Oey., 2012; Thakur et al., 2020). Processing methods such as boiling, roasting, drying, and frying have been observed to enhance bioaccessibility significantly (Victoria-Campos et al., 2015; Thakur et al., 2020).

Cooking methods of food can influence bioactive compounds' bioaccessibility, mainly through change and disruption in the cell wall structure, leading to the release of these compounds, which implies higher bioaccessibility (Victoria-Campos et al., 2015). A few studies have reported the bioaccessibility of bioactive compounds in mixed diets, although only limited information is available. Potatoes and peppers are some of the most frequently consumed food sources globally, providing significantly greater amounts of bioaccessible bioactive compounds (Platel and Srinivasan et al., 2016). There is a significant variation of phytochemical compounds such as phenolics, flavonoids, anthocyanins, and carotenoids among potato cultivars carotenoids (Perla et al 2013.; Kalita and Jayanty., 2014). These compounds exhibited significant antioxidant, antiglycemic, antiviral, anticarcinogenic, and anti-inflammatory activities and showed antiallergic and antimicrobial properties (Sora et al., 2015; Kalita et al 2014).

Potato and peppers are often consumed as part of an elaborate meal, including such ingredients such as salt, fiber, protein, and fat. Thus, it is possible that these ingredients influence

the bioaccessibility of bioactive compounds. This study investigates the bioaccessibility of phenolics, flavonoids, carotenoids, and capsaicinoid compounds in cooked potato cultivars mixed with two roasted pepper varieties via in vitro digestion experiments.

2.Materials and Methods

2.1. Chemicals

Folin Ciocalteu Reagent (FCR), sodium carbonate, gallic acid, potassium chloride, sodium acetate, quercetin, Lutein, and all digestive enzymes were purchased from Sigma-Aldrich. All the reagents are HPLC grade. HPLC grade >90% Purity, Butyl Alcohol, LC, and GC grade Methyl-t-ethyl ether (MtBE), HPLC grade Acetonitrile, HPLC grade, phosphate buffer 0.1 M, 0.1 N HCl, phosphate buffer 0.1 M, pH=7.5, 0.2 M HCl-KCl pH=6.9, α-amylase, pepsin, pancreatin, and porcine bile extract.

2.2. Pepper and potato cultivars

Peppers in this study were sourced from Arkansas Valley Research Center, Rocky Ford CO (AVRC) in July 2016. Two different pepper varieties, Joe Parker (JP; hot) and Sweet Delilah (SD; sweet), were used in this study. Pepper fruits were transported to the San Luis Valley Research Center, Center CO (SLVRC), after harvest to complete the bioaccessibility experiments. Four different potato cultivars: Purple Majesty (PM; purple flesh), Yukon Gold (YG; yellow flesh), Rio Grande Russet (RG; white flesh), and a numbered line (CO 97226-2R/R (R/R; red flesh) were used in this study. The potato cultivars were harvested at the end of the 2015 growing season at SLVRC.

2.3. Cooking methods

Five to six pepper pods of each variety were washed, dried, and cut into small pieces with peduncles removed, then placed on an oven tray, transferred to a preheated oven set at 150 °C, and

roasted for 20 min in a conventional oven. The oven had been preheated for uniform heat distribution. Once removed from the oven, all sample pieces were cooled, freeze-dried (LABCONCO, New York, NY, USA), ground, and stored at −20 °C until further analysis. Five potato tubers from each cultivar were collected at random, and the tubers were pierced twice on each side with a fork and baked at 204 ◦C for one hour in a commercial oven. All potato samples were then cut into small pieces, freeze-dried, ground using a coffee grinder, and stored at −20 °C until further analysis.

2.4. Extraction and estimation the total phenolics and flavonoids

Total phenolics and total flavonoids were extracted with pure methanol. To 15 mL of 100% methanol, 0.4375 g of potato freeze-dried powder and 0.0625 g of roasted pepper freeze-dried powder were added and homogenized for 5 min. Supernatants of pepper and potato extract were filtered to evaluate total phenolics and total flavonoids. The total phenolic content was calculated according to the method declared by (Kalita and Jayanty., 2014) with modifications. FCR solution was added to pepper and potato extract, and sodium carbonate was added to the 96 microplates. The total phenolic content of pepper and potato samples was calculated as gallic acid equivalents (µg/g). A colorimetric method was used to evaluate the total flavonoid content in pepper samples. Aluminum chloride was added to the pepper extract in 96 microplates. The total flavonoid content of pepper samples was expressed as quercetin equivalents (µg/g).

2.5. Extraction and estimation the total carotenoids

Water-saturated butanol was used to extract the total carotenoids from samples. 0.4375 g of potato cultivars and 0.0625 g of roasted peppers were mixed with 15 ml (water-saturated butanol) and homogenized for 5 min. Samples were standing and covered with aluminum foil, in the hood for 60 minutes at room temperature. The absorbance of the contents was measured at

450 nm. Lutein was used as the standard, and the total carotenoid values were quantified as μg of lutein equivalent per gram of dry weight materials using a 5-point calibration curve with an R^2 value of 0.996.

$$\text{Total Carotenoids (mg/g)} = \frac{\left(\frac{\text{Sample Abs - y intercept}}{\text{slope}}\right) \text{ x Orig Vol (L) x } \frac{\text{Dilution}}{\text{Factor}}}{\text{Sample Weight (g)}}$$

where:
Sample Abs = Sample Absorbance
Orig Vol = Original Volume for sample preparation, in liters
Slope = Slope from standard curve

2.6. Extraction of Capsaicinoid Compounds

Capsaicinoid compounds were extracted, and quantification as described by Collins et al., (1995). Samples were added to a 15 mL tube. Then, 10 milliliters of methanol was added to each sample and kept in a shaker overnight at 25 °C. Then, the supernatant was collected and transferred to a new 15 mL tube to collect the first supernatant. Ten milliliters of methanol was added to the residue and shaken as just described. Then two supernatants were combined. Pepper extracts were filtered through 0.45 μm filter and put in a 1.8 mL sample glass vial for high-performance liquid chromatography (HPLC) analysis.

2.7. Analysis of Capsaicinoid Compounds

Capsaicinoid compounds were measured using a Waters HPLC system equipped with a fluorescence detector and C18 column. Two mobile phases A (10% methanol and B 100% methanol) were used with a flow rate 0.4 ml/min. The fluorescence detector was used with excitation and an emission wavelength 280 and 338 nm respectively. Levels of capsaicinoid compounds were evaluated using a calibration curve with a standard of capsaicin and dihydrocapsaicin with concentrations ranging from 0.1 to 10 μg/mL. The concentration of

capsaicinoid compounds was calculated following the standard curve for capsaicin and dihydrocapsaicin.

2.8. In-vitro Digestion.

The in vitro digestion protocol described by Miranda et al., (2013) was performed with modifications in triplicate. In a 50 mL polypropylene tube, 0.4375 g of potato cultivar and 0.0625 g of roasting peppers were mixed with 5 mL of distilled water and homogenized by vortex for 30 s. For salivary digestion, samples were treated with 1 mL α-amylase solution with enzymatic activity 24.0–36.0 U/mg (70 mg/mL in phosphate buffer of 0.1 M pH = 6.9) at 37 °C for 10 min. Sample pH was adjusted to pH 2 with 0.1 M HCl. Samples were then treated with 0.3 mL pepsin with enzymatic activity ≥ 3200 U/mg (300 mg/mL HCl-KCl, 0.2 M pH 1.5) in a 37 °C water bath to complete the gastric digestion phase. Samples were treated with pancreatin with enzymatic activity as markers (5 mg/mL, phosphate buffer, 0.1 M, pH 7.5) and 3.3 mL porcine bile extract (17.5 mg/mL phosphate buffer, 0.1 M, pH 7.5) in a 37 °C water bath to complete the intestinal digestion phase. The resulting digestates were centrifuged at 5000× g for 20 min. Supernatants were collect and stored at −80 °C until further analysis. The bioaccessibility of bioactive compounds in this study is expressed as the percentage of bioactive compounds transferred to the aqueous phase during the in vitro digestion process (amounts of total compounds in the aqueous phase/amount compounds in the sample × 100).

3. Statistical analysis

All experiments were carried out in triplicates and the data were subjected to analysis of variance (ANOVA), and Tukey's test was performed to determine whether differences between

groups were significant at $P < 0.05$. All statistical analyses were performed with R software version 3.4.3 for Windows. All results were presented as mean ± standard deviation (SD) values.

4. Results and discussion

4.1 Total phenolics and bioaccessibility

Vegetables and fruits are the primary sources of dietary polyphenolics, such as phenolic acids, hydroxycinnamic acid derivatives, and flavonoids. These compounds provide many nutritional and health benefits, including antioxidant, anti-inflammatory, and antimicrobial activities (Cartea et al., 2011; Kalita et al., 2013; Sathuvalli et al., 2018). Thus, phenolic compounds are recognized as rich sources of dietary antioxidants (Kumar et al., 2009). However, their health benefits correlate with their bioaccessibility (Zeng et al., 2016). Phenolic compounds such as chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, caffeic acid, p-coumaric acid, and ferulic acid are present at high concentrations in colored flesh potato cultivars and quercetin, luteolin, and capsaicinoids in pepper varieties; however, the bioaccessibility of these compounds can be highly variable. The levels of phenolics and bioaccessible phenolics in potato cultivars mixed with roasted peppers are shown in Figure 4.1. Red-fleshed potato selection R/R mixed with roasted JP had the maximum amount of phenolics (2164 µg/g) followed by PM (1621 µg/g), YG (644 µg/g), and RG (339 µg/g). Similar levels of phenolics (2316 to 329 µg GAE/g FD) in the same order were obtained when mixed with roasted SD (Table 3.1). Higher levels of total phenolics were found in red and purple-fleshed tubers than in white and yellow cultivars (Kalita and Jayanty 2014). These differences in total phenolic content could be attributed to various factors, including the variety, flesh color, starch content, and maturity stages (Kunyanga et al., 2012).

After in vitro digestion, a significant reduction of total phenolics was observed in all samples, which agrees with an earlier report by Andre et al. (2015). The bioaccessible phenolic content was 1316, 869, 485, and 252 µg/g in R/R, PM, YG, and RG potatoes, respectively, when

mixed with JP (Figure 4.1a, and Table 3.1). Our results indicate that 53–75% of phenolic compound content was released from potato cultivars mixed with JP; by contrast, the release was 53–88% in potatoes cultivars mixed with SD (Figure 4.1b and Table 3.1). Our results show that the highest release of the phenolic compounds was observed in YG mixed with SD, whereas the lowest release of phenolic compounds was observed in PM mixed with JP. During gastric digestion, phenolic compounds were highly bioaccessible; 43.8–93.73% of phenolic compounds were released (Vulic´ et al., 2019) . Several reports have established that the bioaccessibility of bioactive compounds is influenced by the composition of the digested food matrix and physicochemical properties, such as pH, temperature, and texture of the matrix (Ryan et al., 2008; Andre et al., 2015).

4.2 Total flavonoids and bioaccessibility

Flavonoids are the most common group of polyphenolic compounds in plants. Flavonoids are natural polyhydroxylated compounds with a proven positive impact on human health. The impact of dietary flavonoids depends on their bioaccessibility. There is little published information on the bioaccessibility of flavonoid compounds following the in vitro digestion procedure. The results in Figure 4.2 show the levels of flavonoids and bioaccessible flavonoids in different potato cultivars either mixed with roasted JP pepper (Figure 4.2a) or with roasted SD (Figure 4.2b). A significant variation ($p \leq 0.05$) was observed in the content of total flavanoids between different potato cultivars mixed with roasted JP (222.4 to 59.25 µg QE/g FD) and SD (277 to 83.9 µg QE/g FD) before digestion (Table 1). Similarly, the bioaccessibility of flavonoid compounds ranged from 185 to 59 µg QE/g FD and from 231 to 64 µg QE/g FD for potato cultivars mixed with JP and SD, respectively. The total flavonoid content and bioaccessibility of flavonoid compounds among the potato cultivars we tested was in the order R/R > PM > RG > YG, irrespective of

peppers in the study (Table 3.1 and Figure 4.2). Our results show that the release of flavonoid compounds ranged from 65 to 83% in potato cultivars mixed with JP, whereas this release ranged from 58 to 83% in potato cultivars mixed with SD. Previous studies have shown that purple and red cultivars had twice the flavonoid concentration of white cultivars (Valcarcel et al., 2015). Various studies suggested that variations in flavonoid levels are primarily the result of the diversity of genotypes, landraces, varieties, and the ripening stage of the fruits (Scarano et al., 2018). Several studies reported that flavonoid bioaccessibility was dependent on the digestible and non-digestible fibers in the tested food product (Tsanova-Savova et al., 2016). Previous studies reported that thermal treatments during food processing increased bioactive compounds' bioaccessibility (Cilla et al., 2018).

4.3 Total carotenoids and bioaccessibility

Carotenoids are a large class of bioactive compounds responsible for the attractive color of many fruits and vegetables. Due to the pro-vitamin A activity, carotenoids constitute a significant source of antioxidants associated with health benefits (Rodríguez-Rodríguez et al., 2020). Carotenoid bioaccessibility depends on the degree of food processing and matrix composition (Fernández-García et al., 2012). Yellow flesh cultivars have generally shown a much higher average of total carotenoid content when compared to red and purple-fleshed potatoes (Pillai et al., 2013). Our studies show that carotenoid levels (194 to 43 µg Lu/g FD) and carotenoid bioaccessibility (152 to 30 µg Lu/g FD) varied in four potato cultivars when mixed with JP (Figure 4.3a). Higher levels of carotenoids were present in YG, and R/R mixed with JP compared to PM and RG. Similarly, total carotenoid levels are between 230 and 49 µg Lu/g FD, and the

bioaccessibility of carotenoids ranged from 185 to 17 µg Lu/g FD in potato cultivars when mixed with roasted SD (4.3b). There are no significant differences in the % release of carotenoids between JP and SD except the cultivar effect (Figure 4.3 and Table 3.1). The differences in the release of flavonoids and carotenoids between JP and SD could be explained by several factors, such as the physical and chemical nature of the food matrix, cooking methods, solubility, and polarity of carotenoids, and interaction with other compounds during the digestion procedure.

Several studies reported that the differences in total carotenoid content among samples have been attributed to variety, maturity stage, and cooking methods, and the presence of other nutrients such as fat and fiber (Venu et al., 2012; Berki et al., 2014; Saini et al., 2015). Andre et al. (2012) reported that the bioaccessibility of lutein and zeaxanthin in the yellow clones ranged from 76 to 82% for lutein and from 24 to 55% for zeaxanthin. The bioaccessibility of carotenoids from raw, frozen, and boiled red chili peppers was studied by Pugliese et al. (2013). They reported that b-carotene and b-cryptoxanthin had lower bioaccessibility, while capsanthin, zeaxanthin, and antheraxanthin had higher bioaccessibility. O'Sullivan et al. (2010) have demonstrated that carotenoid bioaccessibility from red bell peppers ranged from 33 to 87%. One report indicated that the percent accessible all-trans-β-carotene in the supernatant phase was significantly higher—between 24 and 41%—without fat and between 28 and 46% with fat Bengtsson et al. (2012). Several studies have indicated that carotenoid bioaccessibility strongly depends on the food matrix characteristics, chemical structure of carotenoids, and thermal treatments during food processing (Rodríguez-Rodríguez et al., 2020). Various studies suggested that cooking methods such as roasting increase the accessibility of carotenoids (Hedre´ et al., 2002).

4.4 Capsaicinoid compounds and bioaccessibility

Capsaicinoid compounds are widely distributed in pungent pepper fruits. They are the primary active component in chili peppers and have known health benefits. Capsaicin and dihydrocapsaicin are the most abundant capsaicinoids in peppers, together constituting about 90% of the total capsaicinoids in peppers Hamed et al. (2019). In recent years, the consumption of pungent components in hot peppers has increased due to their associated benefits to human health Bley et al. (2012). Figure 4.5 shows a chromatogram of capsaicinoids in JP.

Little information is available in the literature on the bioaccessibility of capsaicinoid compounds in mixed diets. It is vital to assess any beneficial effect when capsaicinoid compounds are added to a carbohydrate-rich diet. The levels of capsaicin and bioaccessible capsaicin in potato cultivars mixed with hot roasted pepper are shown in Figure 4.5. The range of capsaicin and bioaccessible capsaicin in potato cultivars mixed with roasted JP was 52.9 to 66.8 µg/g FD, whereas the levels of dihydrocapsaicin and bioaccessible dihydrocapsaicin in potato mixed with roasted JP ranged from 22 to 13 µg/g FD, whereas potato cultivars mixed with roasted SD did not show capsaicin and dihydrocapsaicin. After in vitro digestion, there was a significant reduction in capsaicin and dihydrocapsaicin in potato cultivars mixed with roasted JP. Similarly, loss of capsaicinoid compounds was observed in jalapeño peppers following heat treatment (Victoria-Campos et al., 2015). The bioaccessibility of capsaicin ranged from 55 to 72%, while dihydrocapsaicin's bioaccessibility ranged from 56 to 83%. Potato cultivar YG mixed with hot roasted pepper had the highest capsaicin bioaccessibility, while cultivar PM had the lowest. The

dihydrocapsaicin bioaccessibility ranking was: RG mixed with hot pepper > YG > R/R > PM. Our in vitro digestion results support those reported by (Victoria-Campos et al., 2015). They found that cooking methods such as boiling and grilling improved the bioaccessibility of dihydrocapsaicin and capsaicin, respectively, in red pepper. For instance, capsaicin bioaccessibility is significantly influenced by the interaction between ripening stage and heat processing, whereas dihydrocapsaicin bioaccessibility is substantially influenced by the interaction between the type of dietary fat, ripening stage, and heat processing (Victoria-Campos et al., 2015).

The interaction of the effects of potato cultivars and roasted peppers on phenolics is shown in Figure 4.6a, flavonoids 4.6b, and carotenoids 4.6c. The interaction bar graph (with a difference as a response) showed that there is evidence of a significant interaction between potato cultivars and roasted pepper bioactive compounds.

5. Conclusions

Peppers and potatoes are the most consumed crops in the world due to culture and eating habits. Significant variations ($p \leq 0.05$) were observed in the levels of bioactive compounds in potatoes mixed with roasted peppers. After in vitro digestion, our results indicate that more than 50% of bioactive compounds are released from the matrix. The present study suggests that phenolics, flavonoids, carotenoids, and capsaicinoids are highly bioaccessible from potato cultivars mixed with roasted pepper varieties. Phenolic compound levels are high in the R/R cultivar, but the highest release of the phenolic compounds was observed in YG mixed with SD, whereas the lowest release of phenolic compounds was observed in PM mixed with JP. Similarly, red flesh cultivars have higher flavonoid levels, and there were differences between JP and SD in % release after in vitro digestion. There were no significant differences in the % release of carotenoids between JP and SD except the cultivar effect.

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6. Figures and Tables

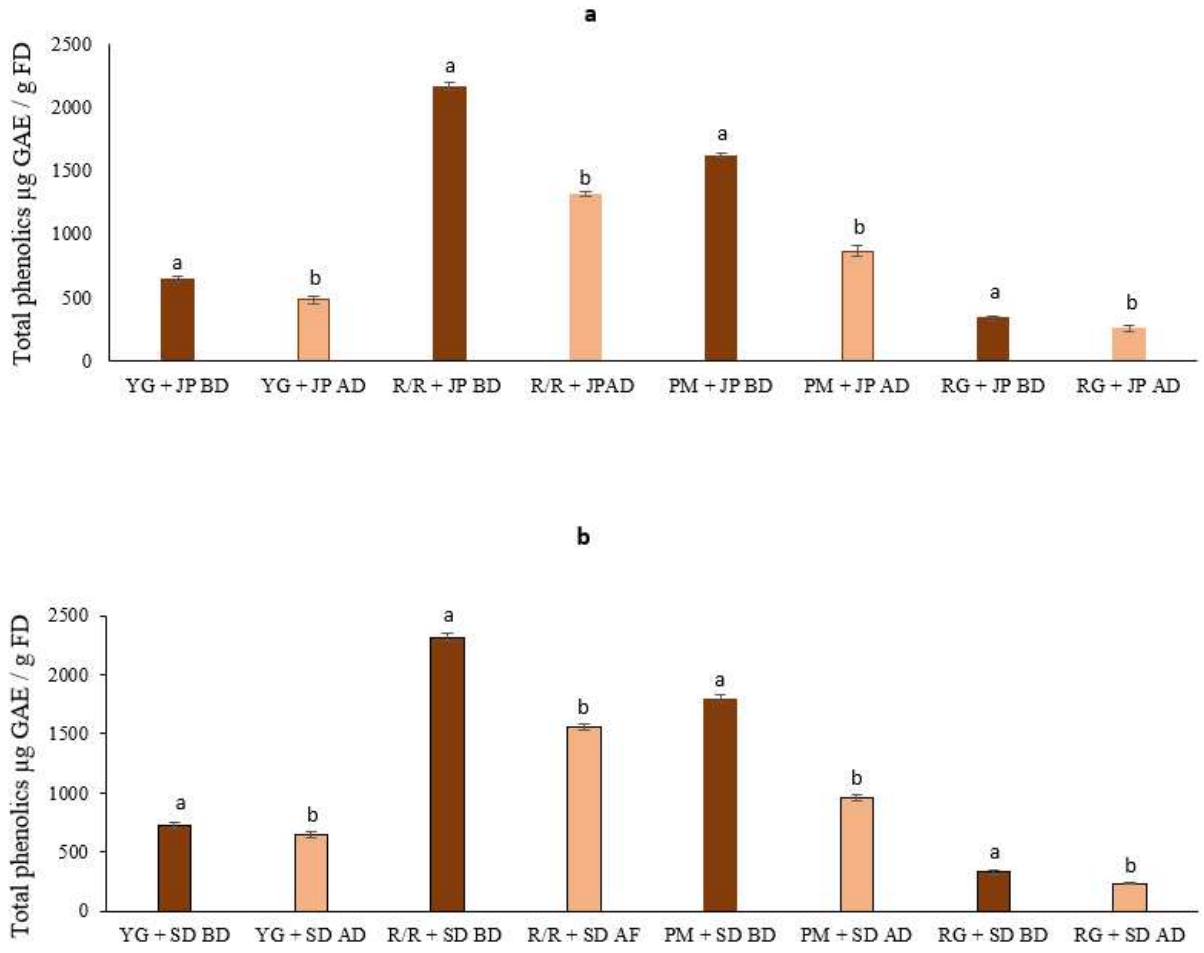

Figure 4.1. The levels of total phenolics and bioaccessibility of cooked potato varieties mixed with roasted JP (a) and with roasted SD (b). Data are the mean of three replicates with standard deviation and are expressed as per gram of freeze-dried weight. Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significantly different. BD and AD: before and after digestion.

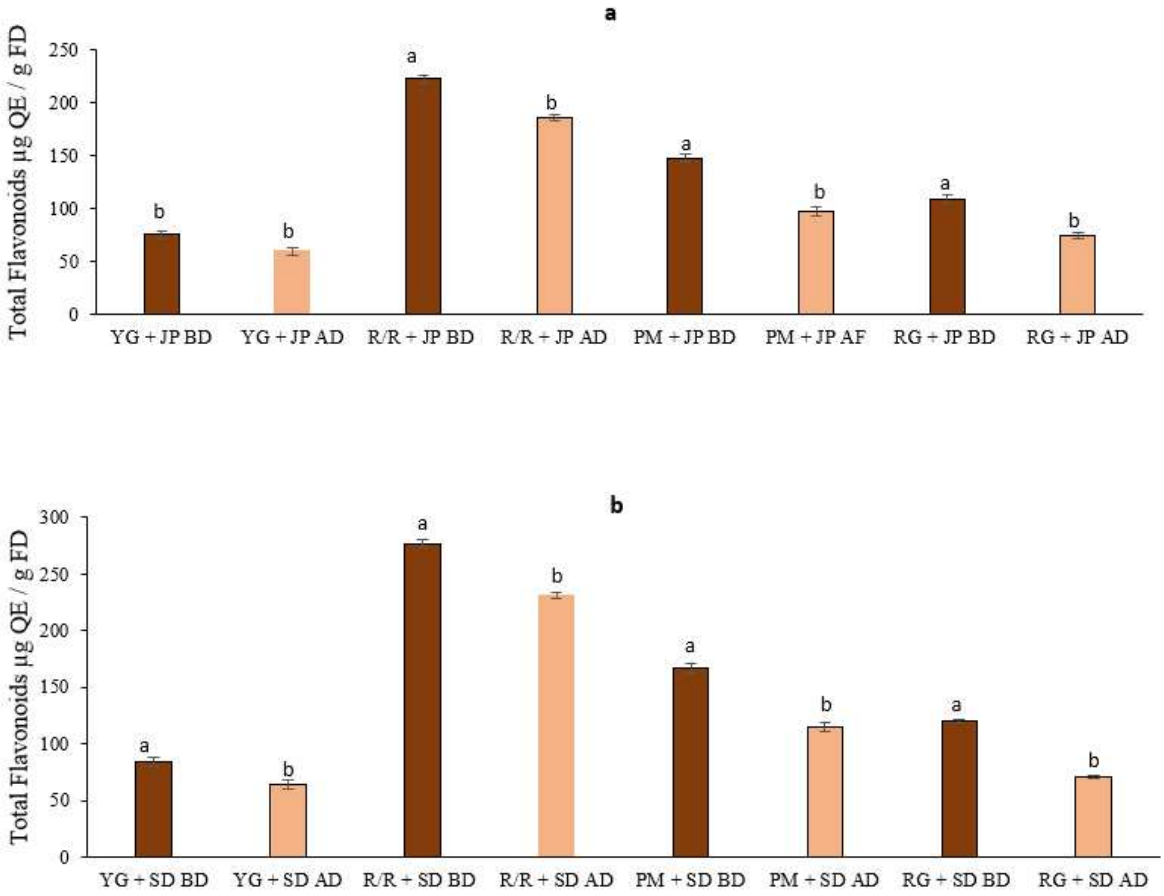

Figure 4.2. The levels of total flavonoids and bioaccessibility of cooked potato cultivars mixed with roasted JP (a) and roasted SD (b). Data are the mean of three replicates with standard deviation and expressed as per gram of freeze-dried weight. Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significantly different. AD and BD: before and after digestion.

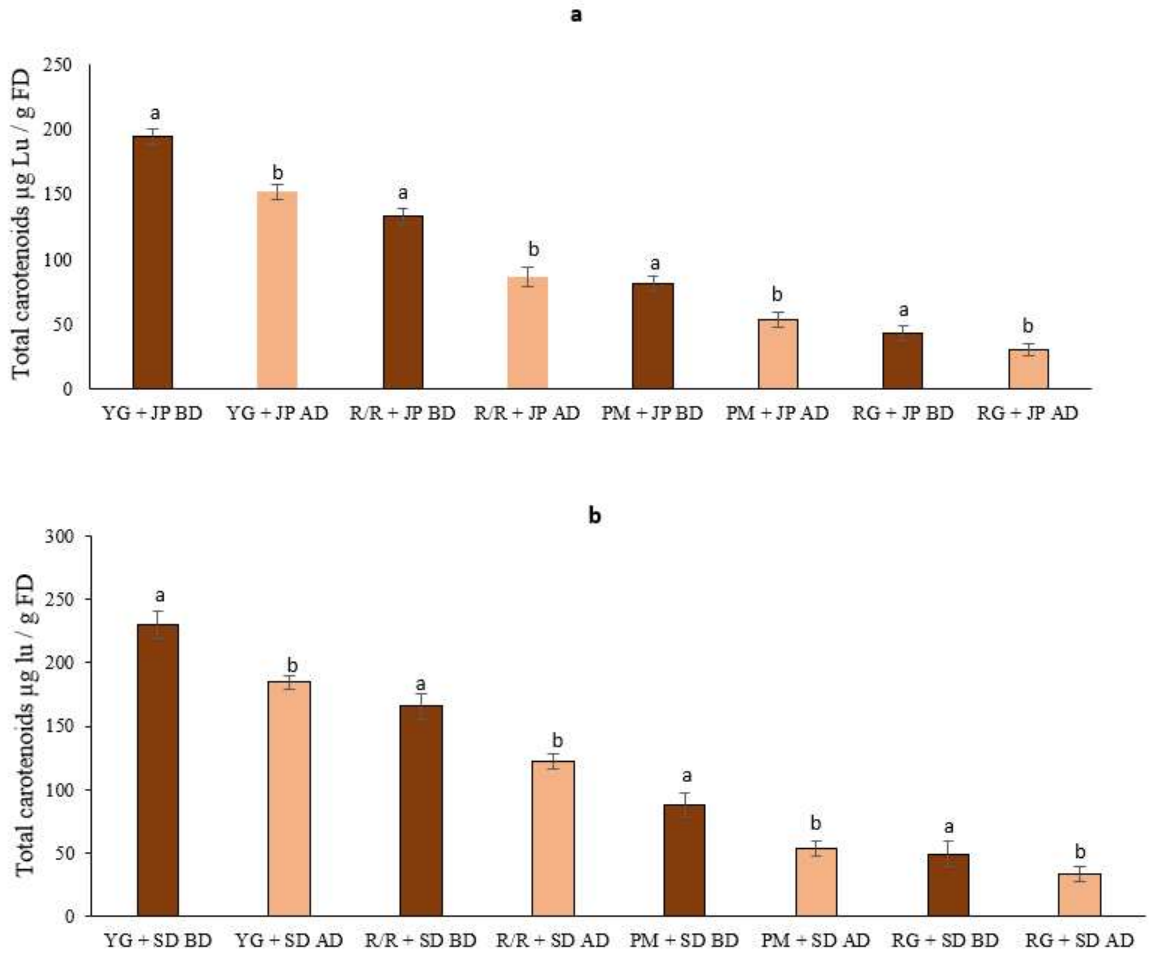

Figure 4.3. The levels of total carotenoids and bioaccessibility of cooked potato cultivars mixed with roasted JP (a) and roasted SD (b). Data are the mean of three replicates with standard deviation and are expressed as per gram of freeze-dried weight. Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significantly different. BD and AD: before and after digestion.

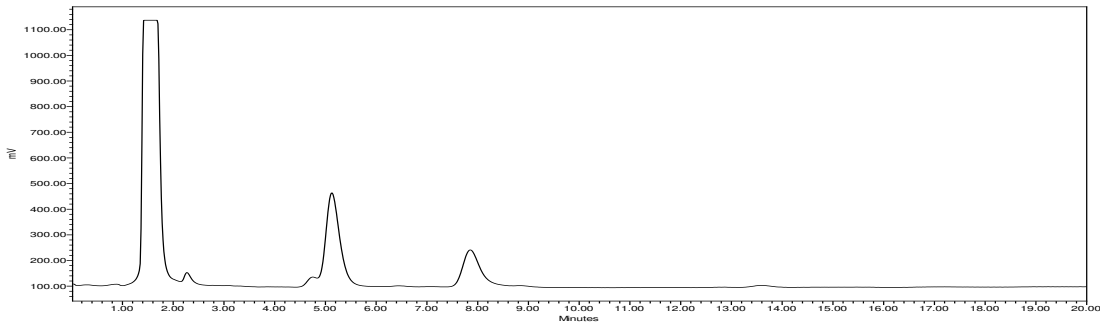

Figure 4.4. A representative HPLC chromatogram of Joe Parker showing baseline separation of capsaicin (A) and dihydrocapsaicin (B)

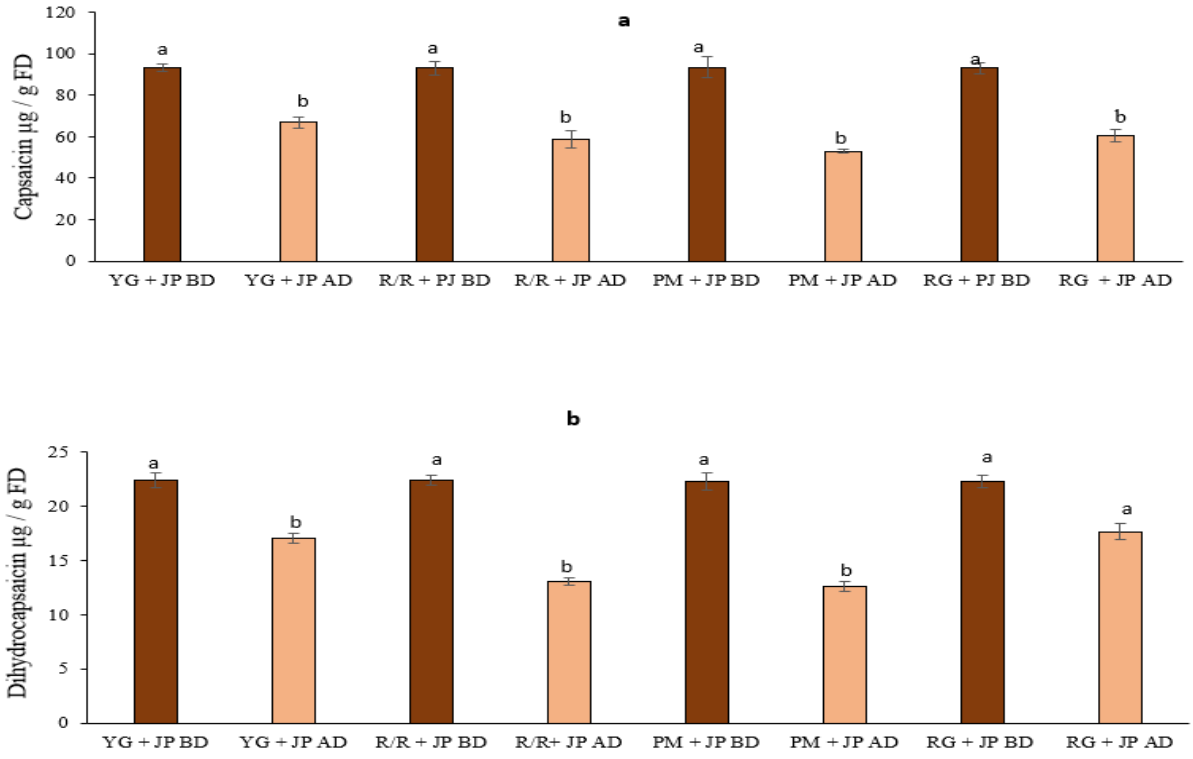

Figure 4.5. The levels of capsaicin and bioaccessibility of capsaicin of cooked potato cultivars mixed with roasted JP (a) and the levels of dihydrocapsaicin and bioaccessibility of dihydrocapsaicin of cooked potato cultivars mixed with roasted JP (b). Data are the mean of three replicates with standard deviation and are expressed as per gram off freeze-dried weight. Significant differences are denoted by different letters, while the same or shared letters indicate that they are not significantly different. BD and AD: before and after digestion

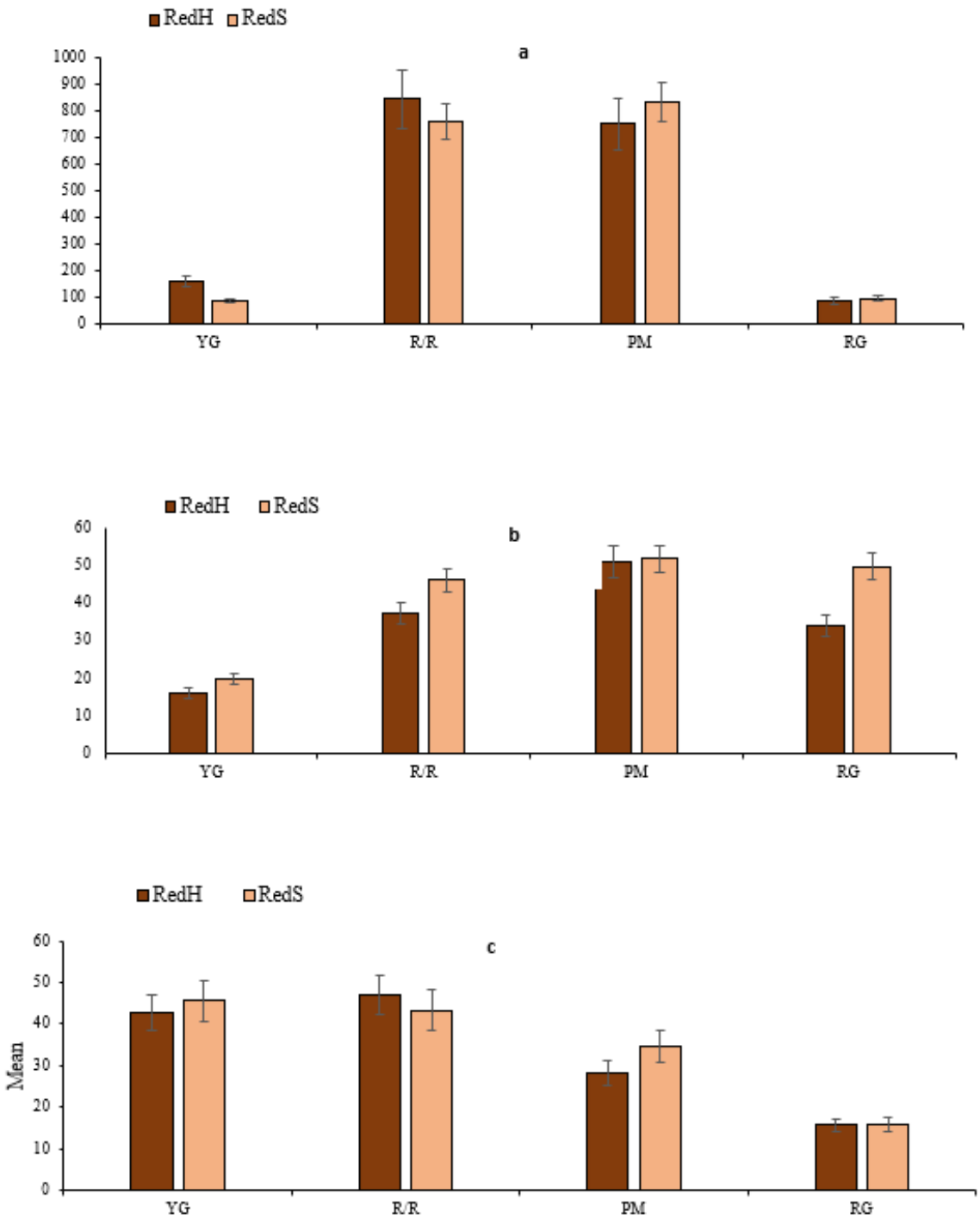

Figure 4.6. (a–c) The interaction bar of cooked potato cultivars with roasted pepper cultivars on bioactive compounds.

Table 3.1. Concentration of total bioactive compounds in potatoes cultivars mixed with two roasted peppers, bioaccessibility, and percentage release during in vitro digestion experiment. Data are the mean of three replicates with standard deviation and are expressed as per gram of freeze-dried weight. Significant differences between bioactive compounds before (^{a}) and after digestion (^{b}) are denoted by different letters, while the same or shared letters indicate that they are not significantly different.

	Bioactive compounds								
Treatment 1	Total phenolic µg GAE / g FD	Bioaccessible total phenolics µg GAE / g FD	% release	Total flavonoids µg QE / g FD	Bioaccessible total flavonoids µg QE / g FD	% release	Total carotenoids µg Lu/ g FD	Bioaccessible total carotenoids µg Lu / g FD	% release
YG + JP	$644.3^{a} \pm 27$	$485.7^{b} \pm 29$	75.3	$75^{a} \pm 3.6$	$59^{b} \pm 4.1$	78.6	$194^{a} \pm 5.6$	$151^{b} \pm 5.6$	78
R/R + JP	$2164.3^{a} \pm 29$	$1316.8^{b} \pm 20$	60.8	$222^{a} \pm 3.5$	$185^{b} \pm 2.9$	83.2	$133^{a} \pm 5.5$	$86^{b} \pm 11.3$	64.6
PM + JP	$1620.8^{a} \pm 19$	$869.06^{b} \pm 42$	53.6	$147^{a} \pm 3.7$	$96^{b} \pm 3.8$	65.5	$81^{a} \pm 5.7$	$53^{b} \pm 2.5$	65.4
RG + JP	$338.7^{a} \pm 20$	$252.7^{b} \pm 22$	74.6	$107^{a} \pm 4.3$	$74^{b} \pm 2.9$	68.6	$42^{a} \pm 5.4$	$30^{b} \pm 1.8$	71
	Bioactive compounds								
Treatment2	Total phenolic µg GAE / g FD	Bioaccessible total phenolics µg GAE / g FD	% release	Total flavonoids µg QE / g FD	Bioaccessible total flavonoids µg QE / g FD	% release	Total carotenoids µg Lu/ g FD	Bioaccessible total carotenoids µg Lu / g FD	% release
YG + SD	$723.6^{a} \pm 19$	$639^{b} \pm 26$	88.3	$83.9^{a} \pm 3.6$	$64.1^{b} \pm 3.5$	76.4	$230^{a} \pm 11$	$184.5^{b} \pm 5.3$	80.1
R/R + SD	$2316^{a} \pm 32$	$1556^{b} \pm 26$	67.1	$277^{a} \pm 3.7$	$231.1^{b} \pm 2.9$	83.4	$165.5^{a} \pm 9.6$	$122.2^{b} \pm 5.6$	73.8
PM + SD	$1794^{a} \pm 29$	$959.9^{b} \pm 28$	53.4	$167^{a} \pm 5.2$	$114.8^{b} \pm 3.3$	68.9	$88.09^{a} \pm 9.1$	$53.4^{b} \pm 5.7$	60.6
RG + SD	$328.7^{a} \pm 20$	$234.6^{b} \pm 11$	71.3	$120^{a} \pm 2.5$	$70.6^{b} \pm 1.5$	58.7	$49.3^{a} \pm 9.3$	$17.3^{b} \pm 2.5$	68.3

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CHAPTER 5: CONCLUSIONS

Peppers (*Capsicum annum L.)* are important crop species around the world for their color, flavor, spice, and nutritional value. Therefore, peppers are often harvested in the immature green and matured red stages depending on the consumers' interest, such as salads, stuffing, roasting, and added flavor in many cooked dishes. Peppers contain a wide range of phytochemicals such as phenolics, vitamin C, carotenoids, anthocyanins capsaicinoids. However, capsaicinoids are the primary group of compounds that give them their characteristic pungent taste. These compounds have been used in the food technology and pharmaceutical industries because of their potential antioxidant, anticancer, and antibiotic properties. In chapter 2, the research aimed to determine the effect of ripening stage and cooking methods on bioactive compounds in different peppers varieties that grow in Arkansas Valley Research Center (AVRC) Colorado. The bioactive compounds such as phenolics, flavonoids, vitamin C, and capsaicinoids of different pepper varieties were evaluated in different maturation stages green, yellow and red. A significant variation in bioactive compounds was observed among pepper varieties. Our results demonstrated that green, yellow, and red varieties contain different phenolics, flavonoids, vitamin C, and capsaicinoids. Therefore, pepper varieties with red and yellow stage had higher levels of total phenolics than the green stage. Scoville Heat Units (SHU) were used to estimate the pungency of hot pepper varieties. There was a significant variation in pungency levels of hot pepper varieties. Our data showed that different levels of capsaicinoid compounds are accumulated among various pepper cultivars grown at AVRC.

Thus, total capsaicinoid compounds change with maturity in peppers. The variations in pungency levels could be ascribed to several factors such as type of verities, growth conditions, and maturity stages. Capsaicinoids were not detected in sweet pepper varieties such as Flavorburst Canario

Sweet Delilah and Aristotle. The bioactive compounds such as phenolics, flavonoids, vitamin C are the major compounds associated with antioxidant activity. Therefore, DPPH and ABTS assays were used to evaluate the antioxidant activity of pepper varieties. All peppers have significant potential to provide antioxidant activities. Due to the difference in bioactive compounds, pepper varieties showed high levels of antioxidant activity in all maturity stages. Therefore, the high antioxidant capacity of peppers verities can be explained by the presence of numerous phenolic and flavonoid compounds. As peppers are consumed after cooking, we tested the content of the bioactive compound after roasting processing. Overall, with a few exceptions, the capsaicinoids content of pepper verities was decreased by roasting cooking. Our findings suggest that the roasting treatment significantly increased the total phenolics, flavonoids of peppers verities. Thus, the increases of bioactive compounds could be attributed to the dehydration of the food matrix and improved extractability of these compounds by cooking methods such as roasting. Finally, pepper varieties developed in (AVRC) were an excellent source of bioactive compounds with high antioxidant activity.

Pepper fruits are perishable products, and they are not suitable for long-term cold storage. Chapter 3, the research examined the effect of different packaging films and 1-MCP on physiological parameters such as weight loss, texture change, color, respiration rate, and bioactive compounds of Sweet Delilah pepper. The influence of packaging films and 1- MCP on the quality characteristics of Sweet Delilah peppers was investigated. In the present study, Sweet Delilah peppers (*Capsicum annuum L.*) were stored in packaging films made of polyethylene and polypropylene for 21 days. Four packaging films and two different maturity stages were tested. Our findings showed that packaged peppers with different films kept at 7.2 °C, presented significantly less weight loss, texture change, and color change than unpackaged peppers. Our

results demonstrated that packaging pepper in different films resulted in greater retention of texture, color, bioactive compounds, and antioxidant activity during storage. Our results have indicated that peppers packaged with films preserved more phenolics and flavonoids compounds and retained higher antioxidant activity than control samples. Therefore, our results indicated that peppers packaged with films should be considered as a more effective method for slowing down the degradation of bioactive compounds. It can be concluded that packaging films could be used to store Sweet Delilah pepper for 21 days under 7.2 °C temperature and 90 % RH condition with the high maintenance of texture, color, and ascorbic acid. Based on the findings of this study, it can be concluded that pepper packaged with films at 7.2 °C storage temperature performed better in slowing down the respiration rate, weight loss, and minor firmness change of packaged peppers compared with unpackaged peppers.

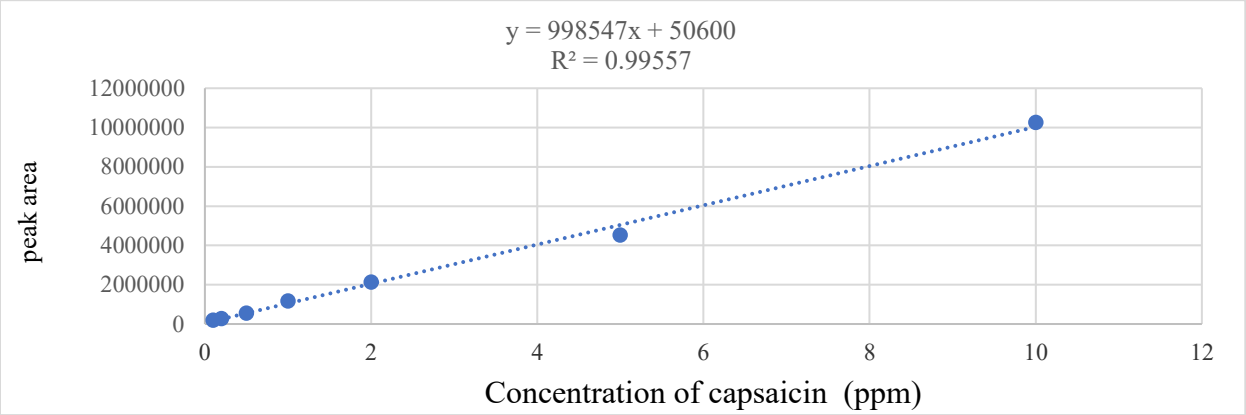
Our results showed that peppers packaged and peppers treated with 1-MCP maintained a higher level of firmness than control samples. Our results indicated that the respiration rate and the ethylene production were decreased in peppers treated with 1-MCP during storage time compared to control peppers. Based on the findings of this study, pepper samples treated with1-MCP had a significant effect in delaying ripening processes and inhibiting color changes. Overall, these results suggested that packaging films and 1-MCP had a significant impact on the peppers' bioactive compounds and antioxidant properties. Our results showed that peppers packaged with different films effectively slowed down the decrease in total phenolic and total flavonoids in both the green and red stages. Using 1-MCP can effectively delay weight loss, color change, maintain firmness, and extend the shelf life of red pepper fruits. Finally, pepper fruits packaged with films and treated with 1-MCP showed significantly better quality and storability potential than control.

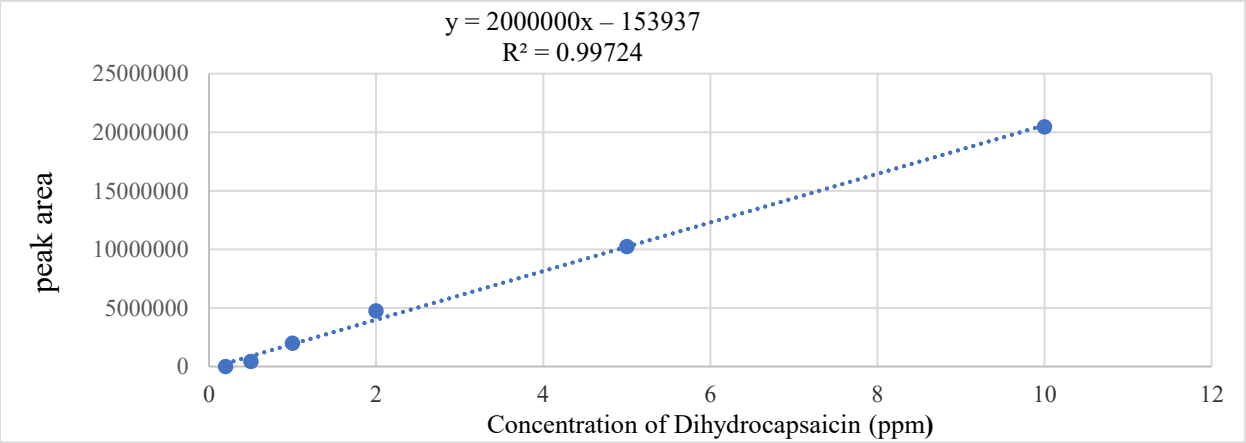
Thus, we concluded that packaging films with different thicknesses and 1-MCP could be used to store pepper varieties with high bioactive compounds.

In Chapter 4, the research estimated the bioaccessibility of phenolics, flavonoids, carotenoids, and capsaicinoid compounds in cooked potato cultivars mixed with roasted pepper varieties in vitro digestion experiment. Our results showed that potato cultivars mixed with roasted pepper varieties contain significantly higher phenolics, flavonoids, and carotenoids. Therefore, a high variation in the bioaccessibility of bioactive compounds was observed in potato cultivars mixed with both hot pepper (JP) and sweet pepper (SD). There was a significant difference between bioactive compounds before and after the digestion process in potato cultivars mixed with pepper varieties. Our findings indicated that bioactive compounds are highly bioaccessible from cooked potato cultivars mixed with roasted pepper varieties. Our results indicated that more than 50% of bioactive compounds are released from the matrix during in-vitro digestion. A high release of bioactive compounds from cooked potato cultivars mixed with roasted pepper verities could be ascribed to the specific gravity of potato cultivars, cultivars, and cooking methods such as roasting. These variations in the bioaccessibility could be described to several factors such as the type of food matrix, the interaction between food matrix, and cooking processing such as roasting and boiling. Finally, Colorado potato cultivars and pepper verities are good sources of phytonutrients viz, phenolics, flavonoids, carotenoids, and capsaicinoid compounds.

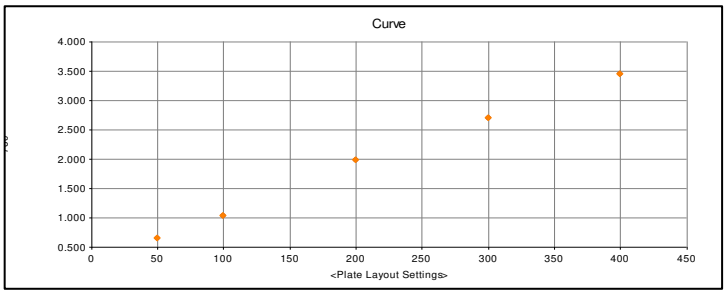
APPENDICES

Supplemental Figures

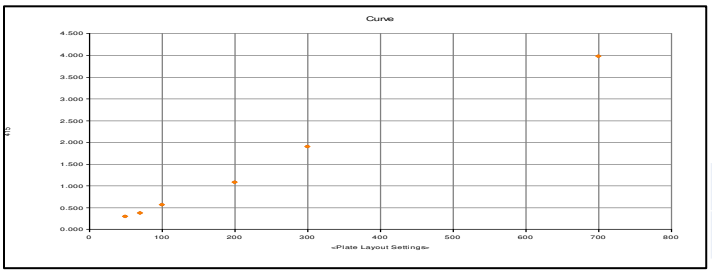




Supplemental Figure S1. Standard curves of capsaicin and dihydrocapsaicin to estimate capsaicinoid compounds.

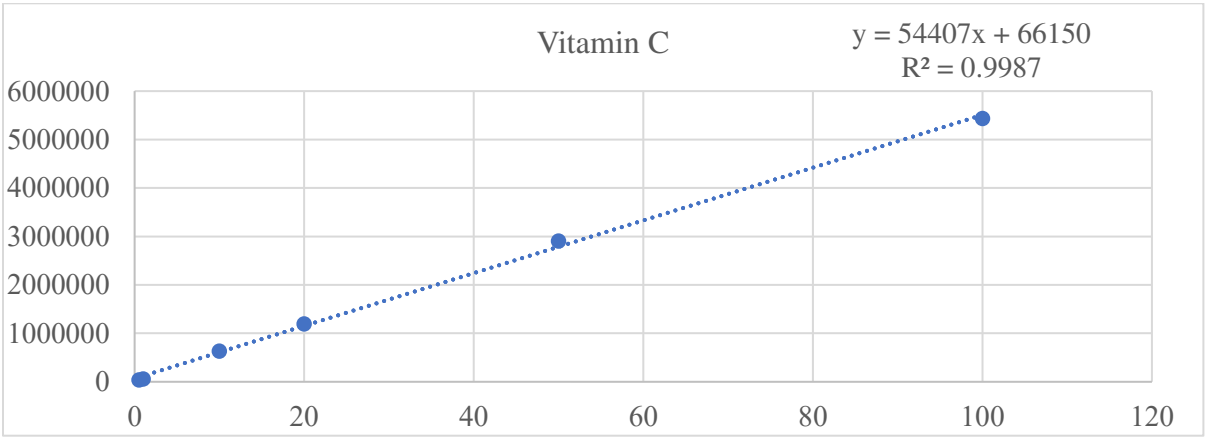


Curve Name	Curve Formula	A	B	R^2
Curve	Y=A*X+B	0.00807	0.278	0.997

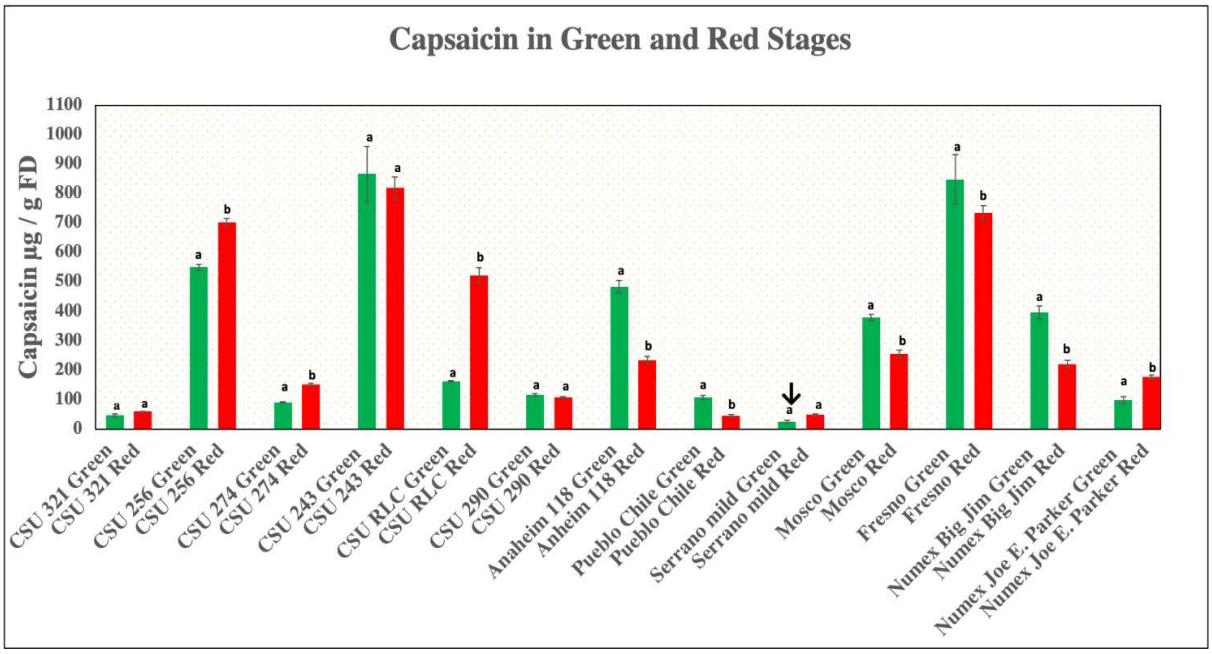


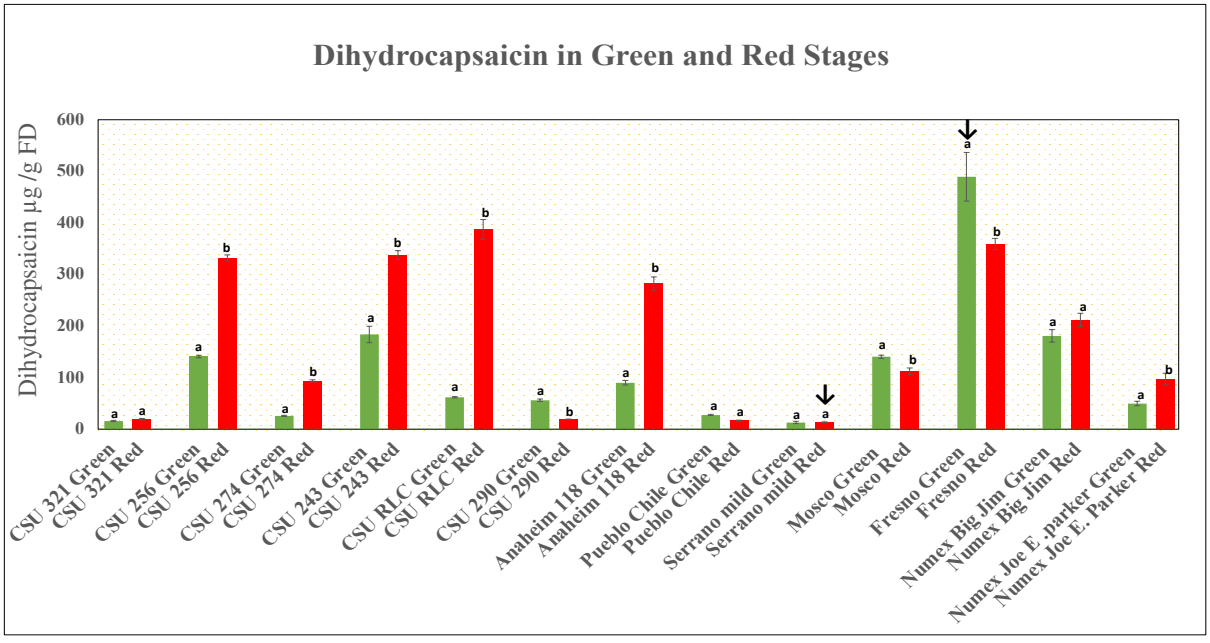
	Curve Name	Curve Formula	A	B	R^2
	Curve	Y=A*X+B	0.00555	0.0897	0.98

Supplemental Figure S2. Standard curve of gallic acid and quercetin to estimate total phenolics and total flavonoids.

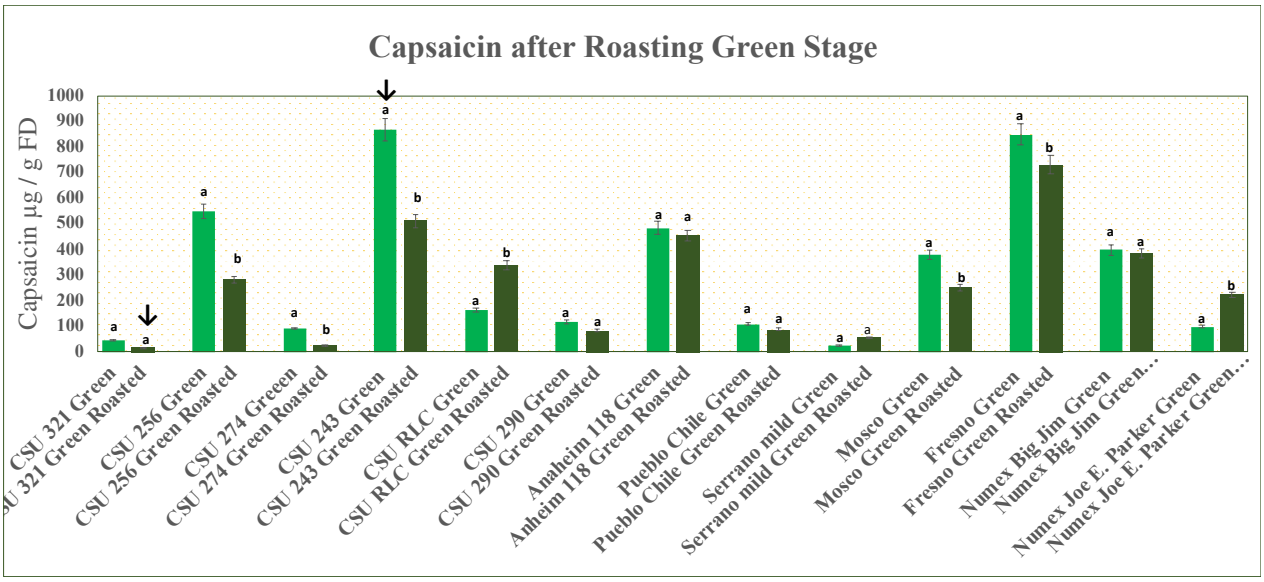


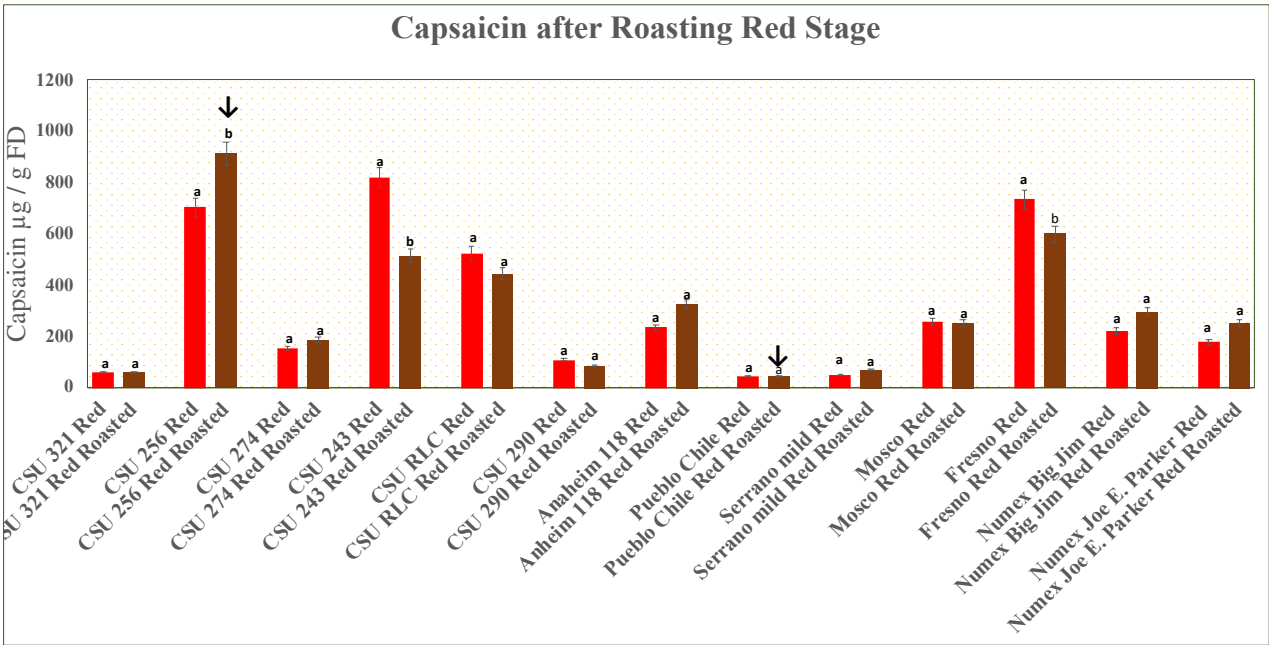
Supplemental Figure S3. Standard curve of ascorbic acid to estimate vitamin C.



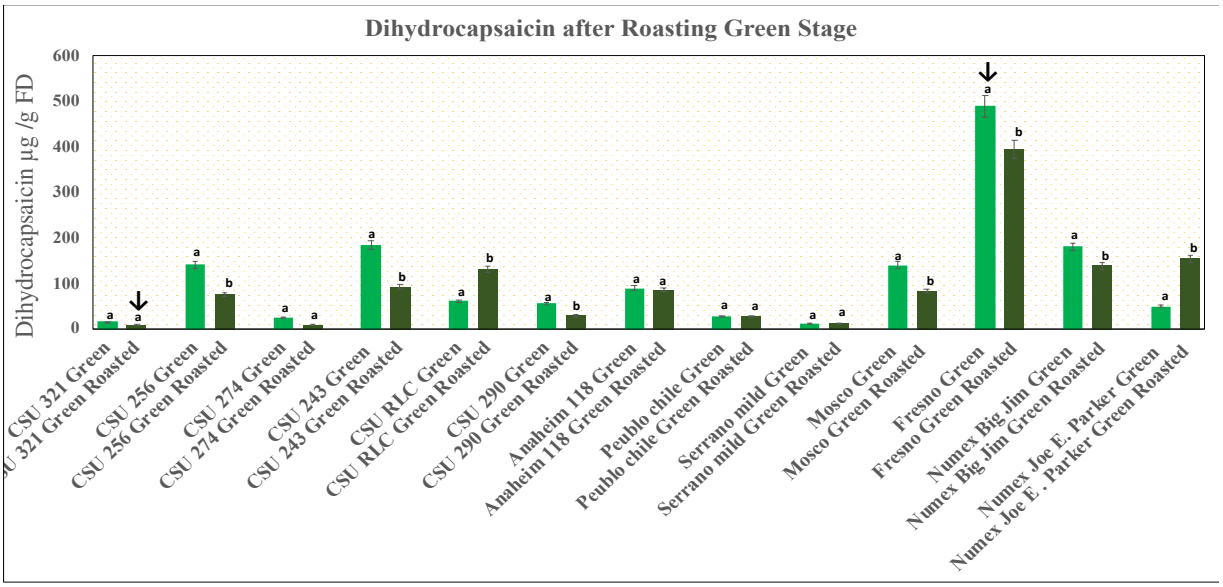


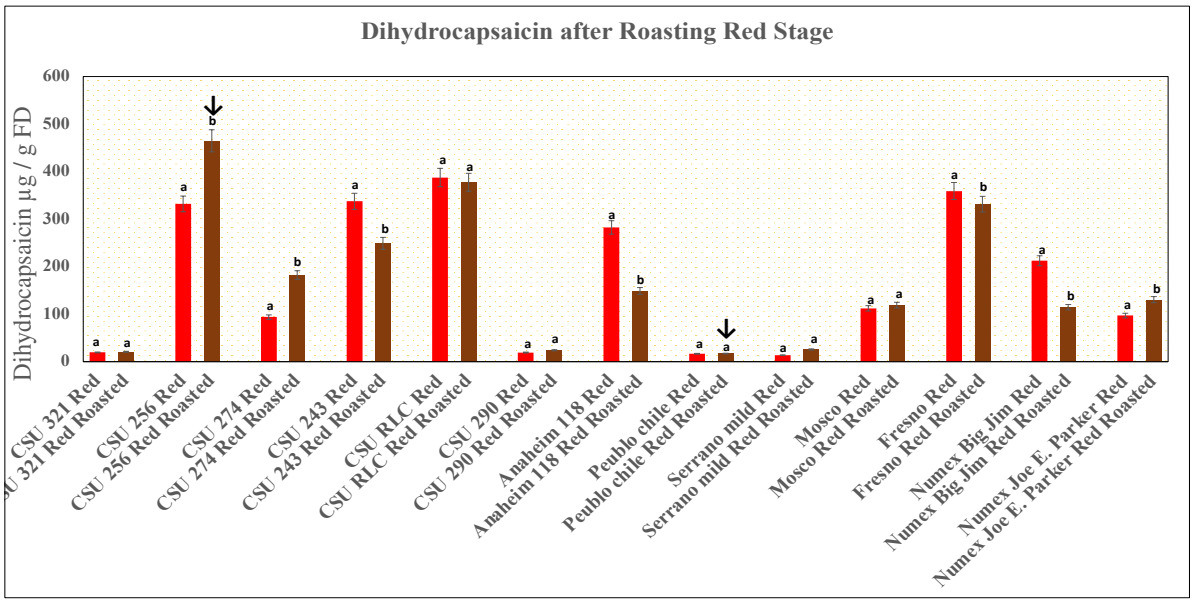
Supplemental Figure S4. Levels of capsaicin and dihydrocapsaicin in pepper cultivars that grown in RVRC in both maturity stages green and red.



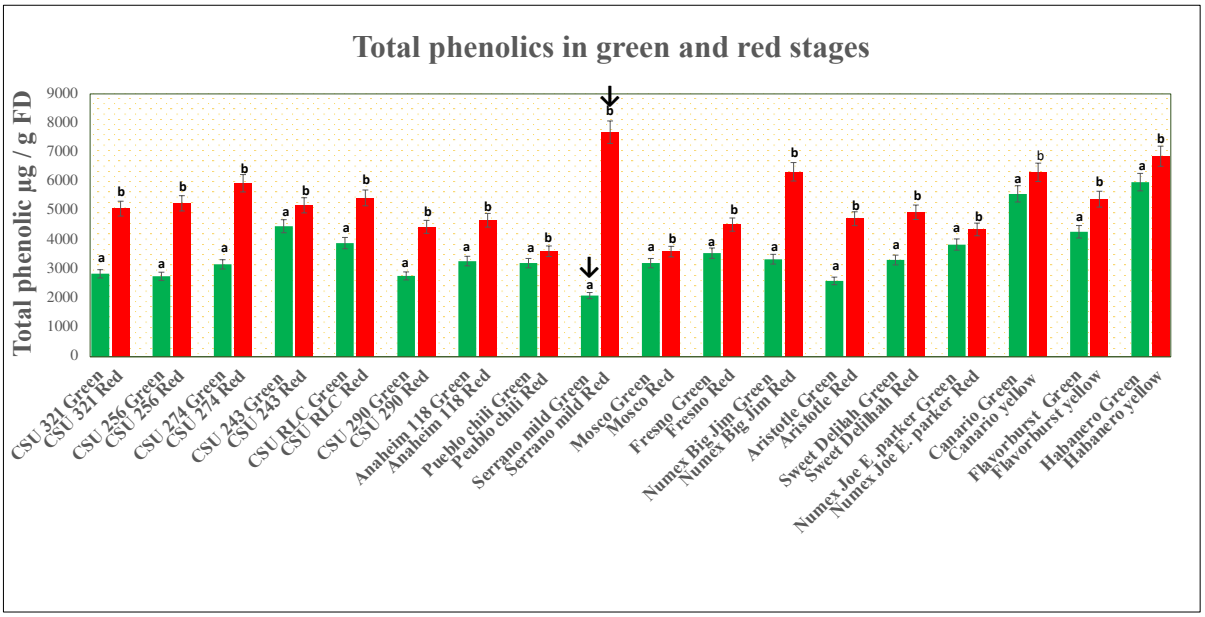


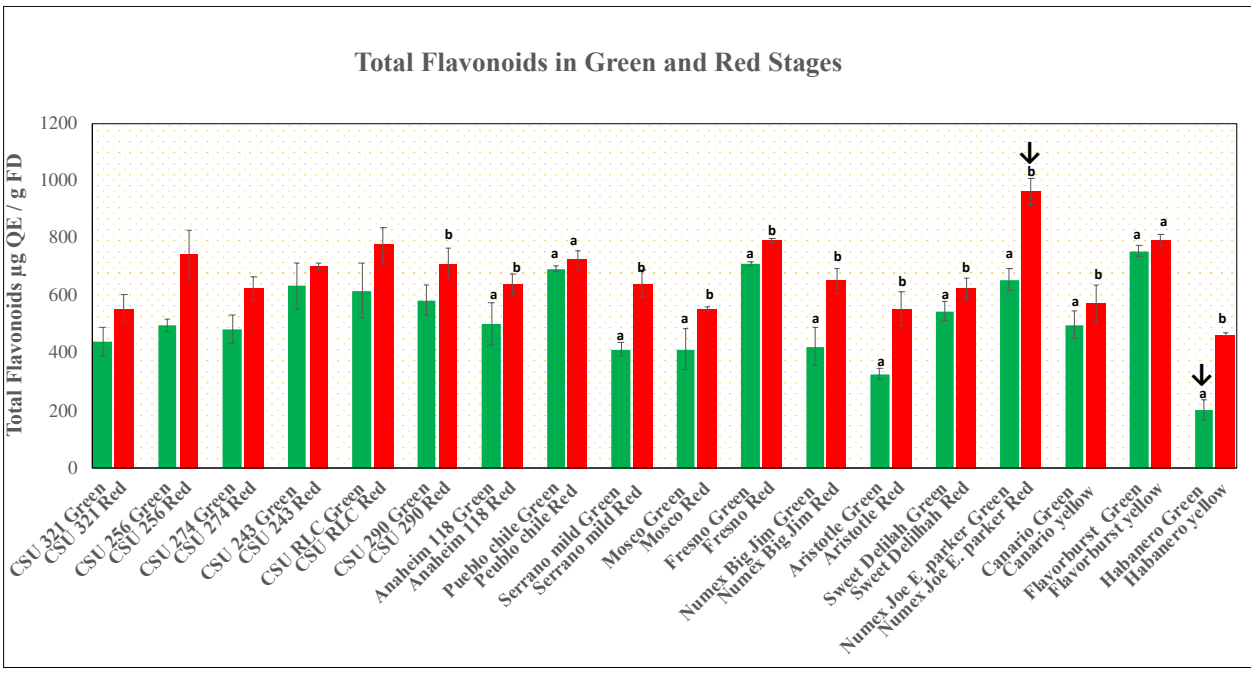
Supplemental Figure S5. Effect of roasting method on capsaicin levels of pepper cultivars that grown in RVRC in both green and red stages.



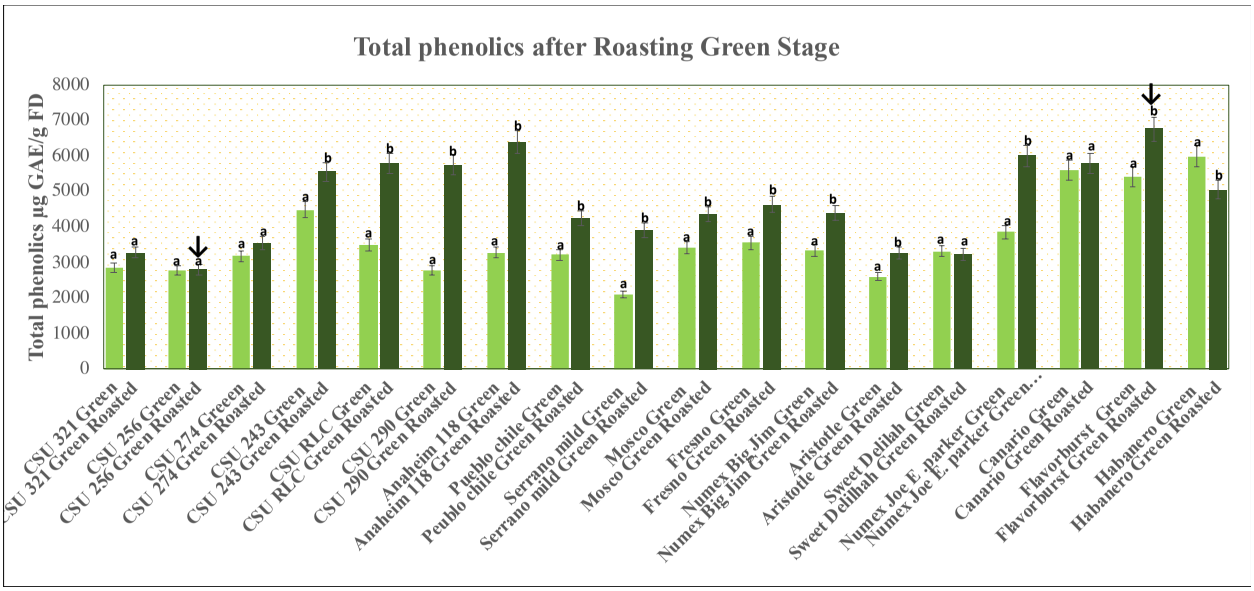


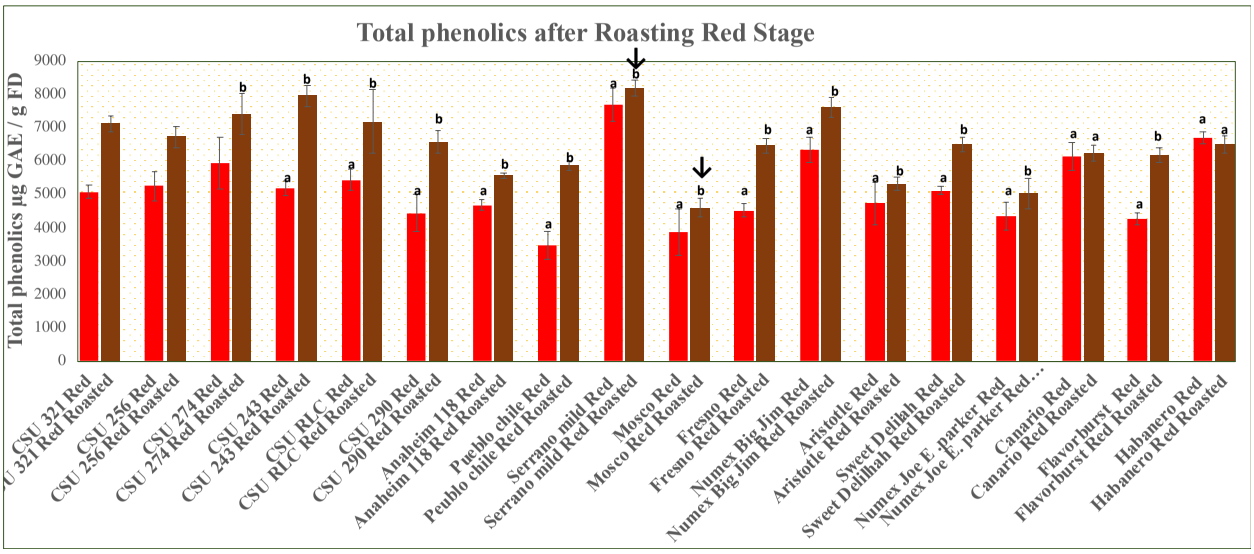
Supplemental Figure S6. Effect of roasting method on dihydrocapsaicin levels of pepper cultivars that grown in RVRC in both green and red stages.



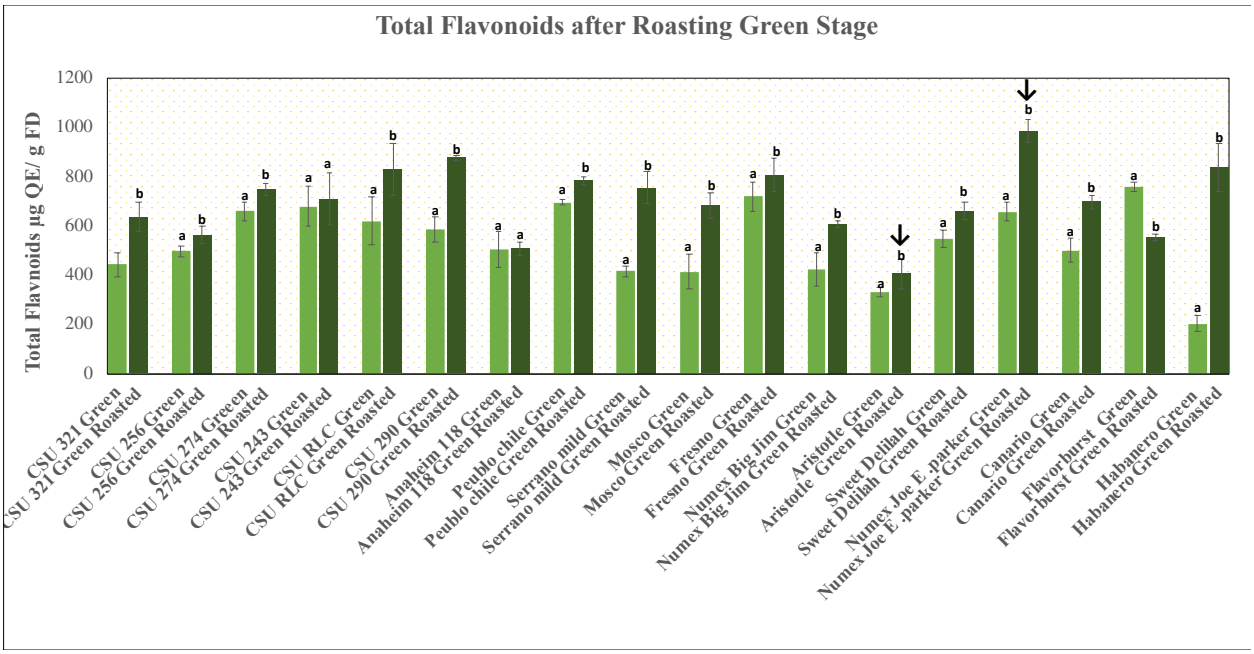


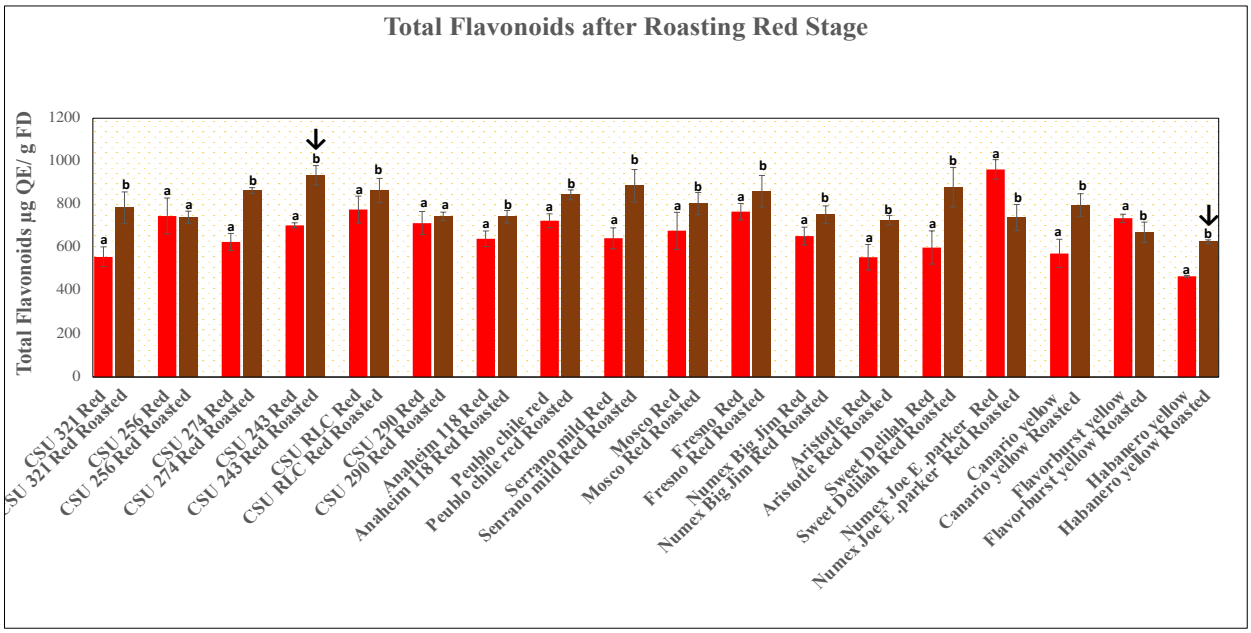
Supplemental Figure S7. Levels of total phenolics and total flavonoids of pepper cultivars that grown in RVRC in both green and red stages.



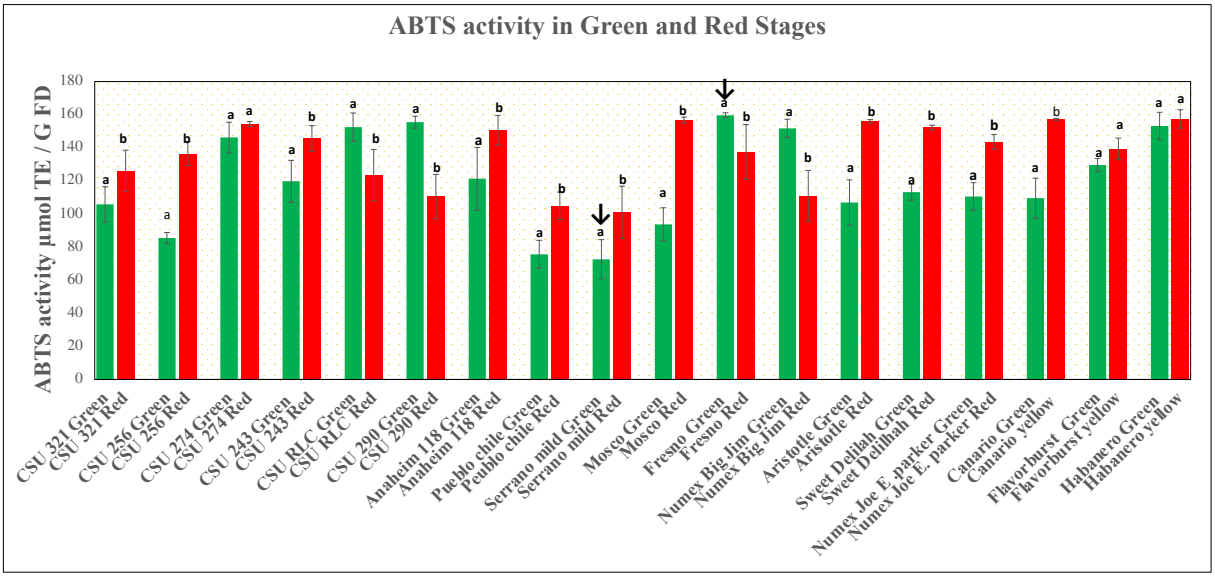


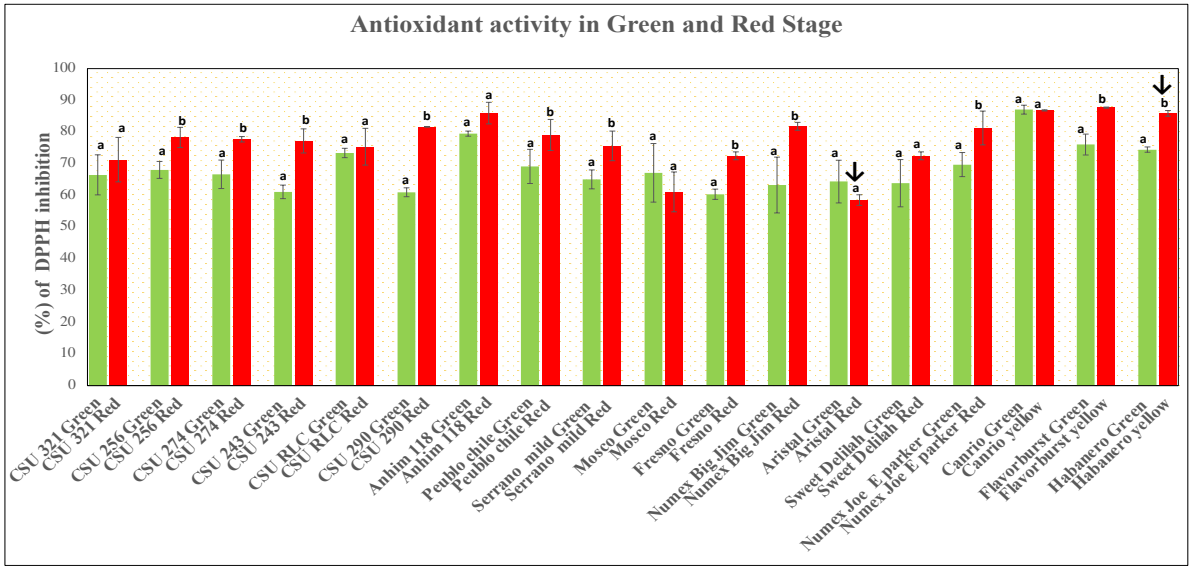
Supplemental Figure S8. Effect of roasting method on total phenolics levels of pepper cultivars that grown in RVRC in both green and red stages.



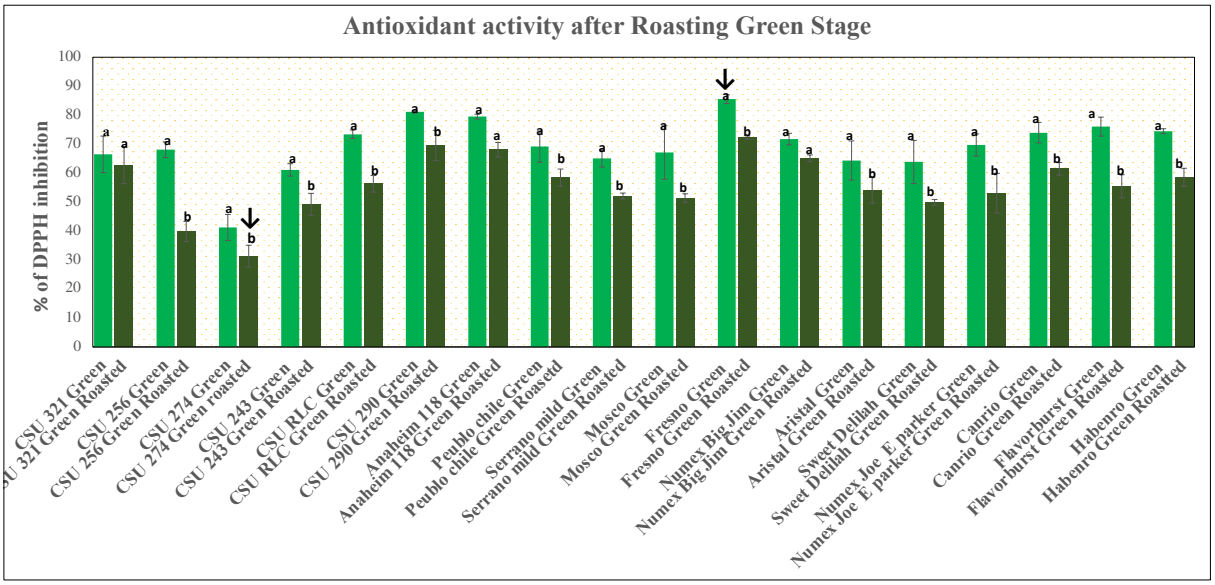


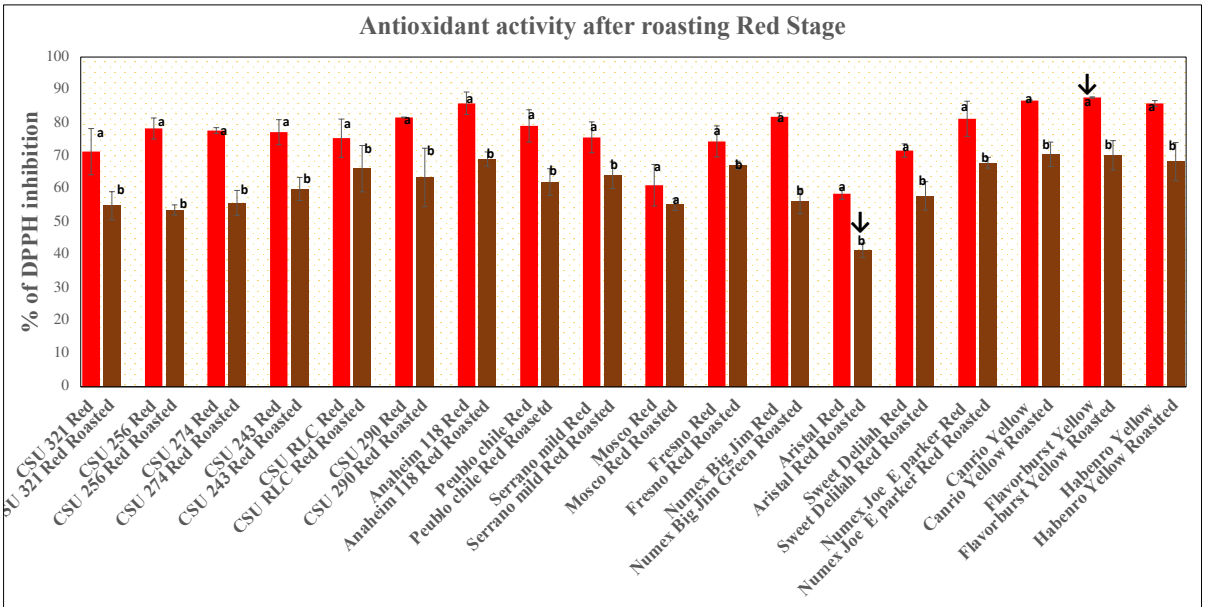
Supplemental Figure S9. Effect of roasting method on total flavonoids levels of pepper cultivars that grown in RVRC in both green and red stages.



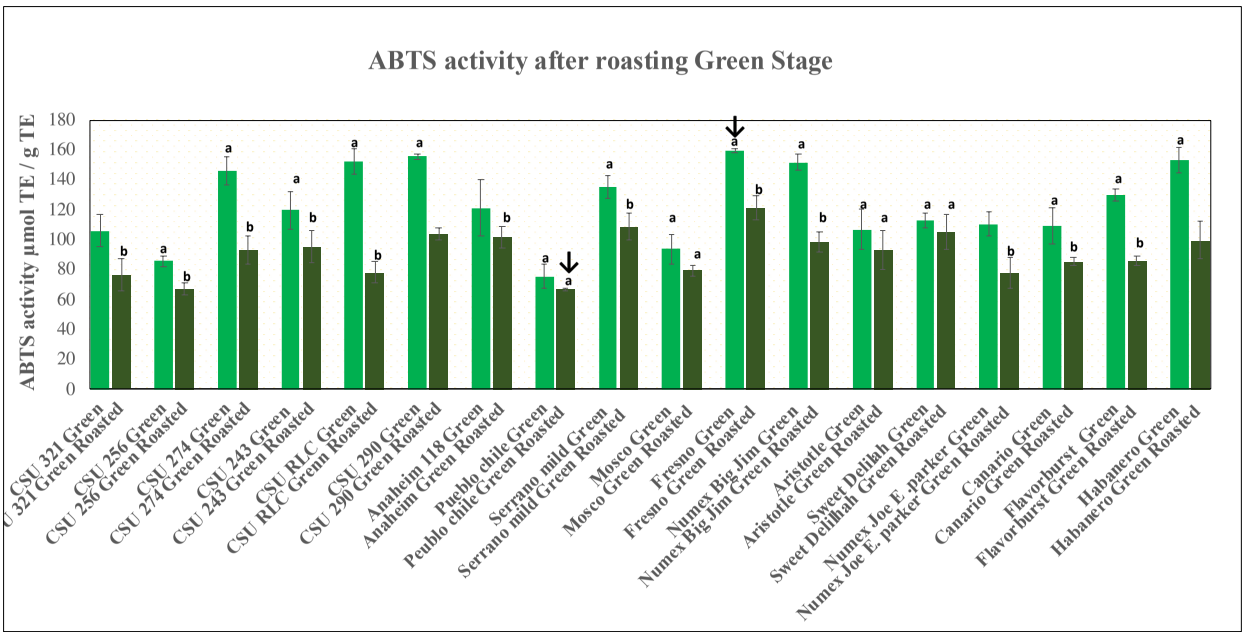


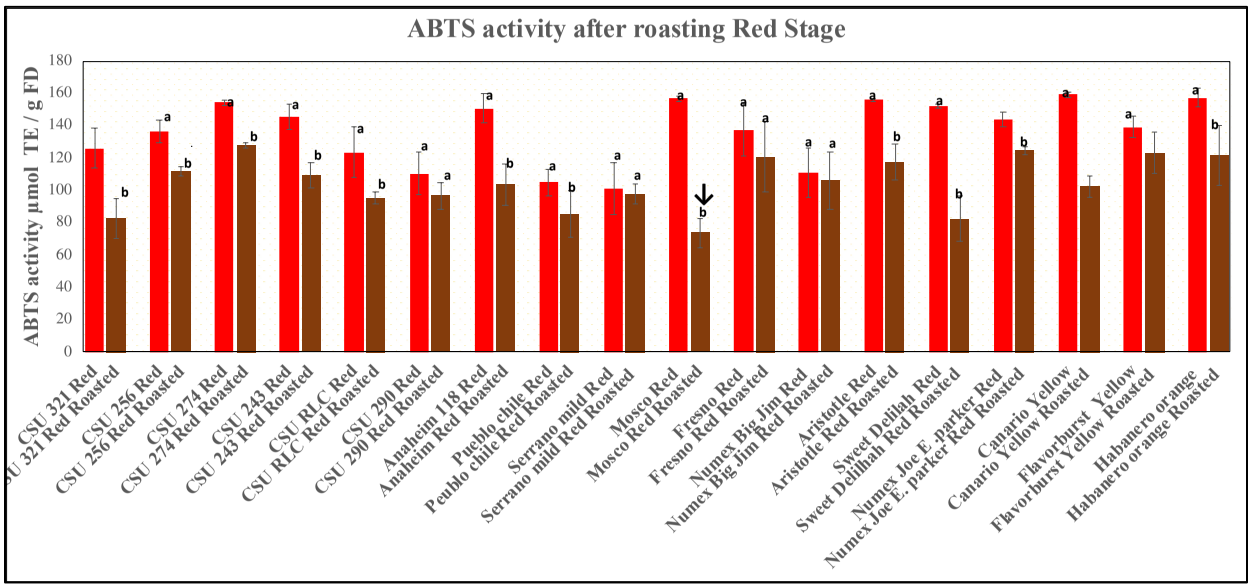
Supplemental Figure S10. Antioxidant activities of green and red pepper cultivars that grown in ARVRC by ABTS and DPPH assay.



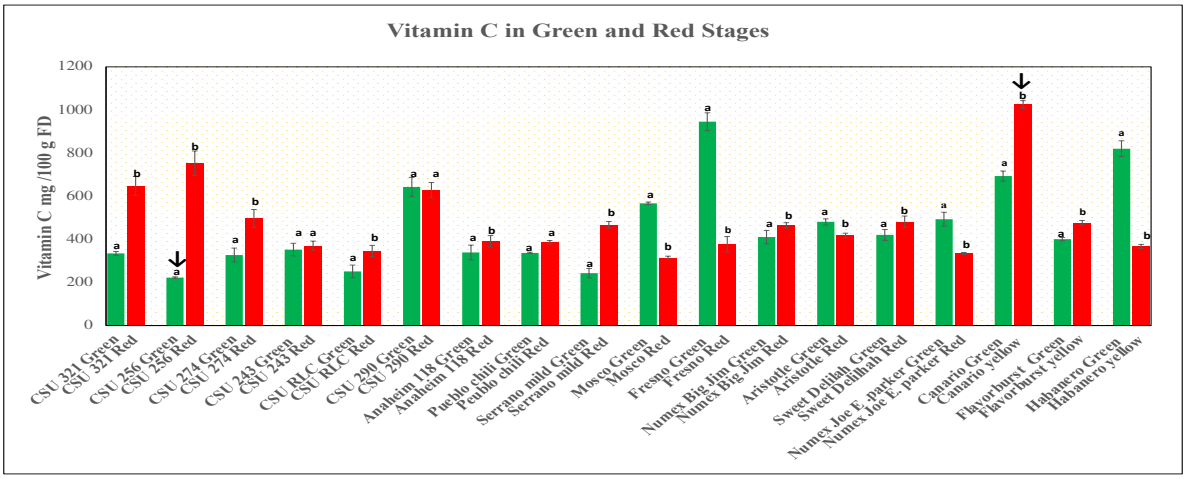


Supplemental Figure S11. Effect of roasting method on antioxidant activities of green and red pepper cultivars that grown in AVRC by DPPH assay.

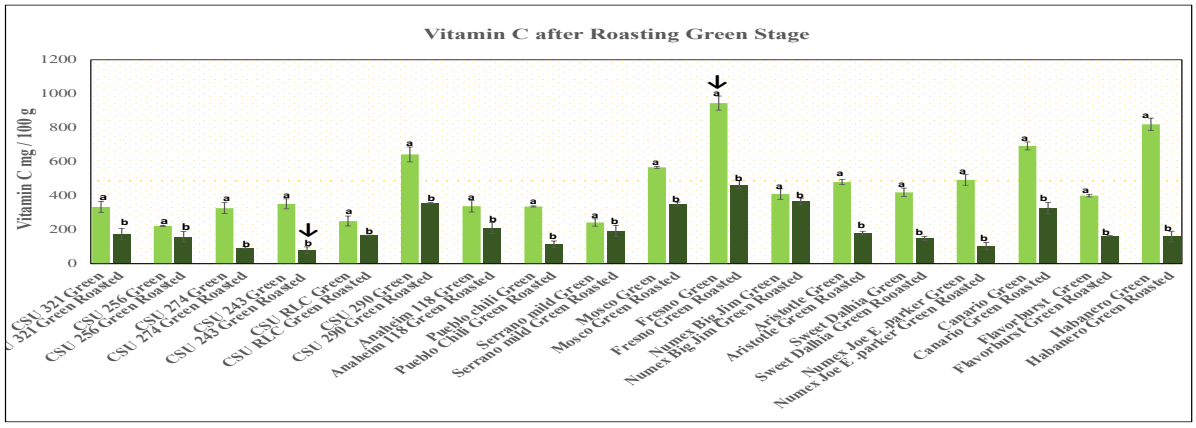


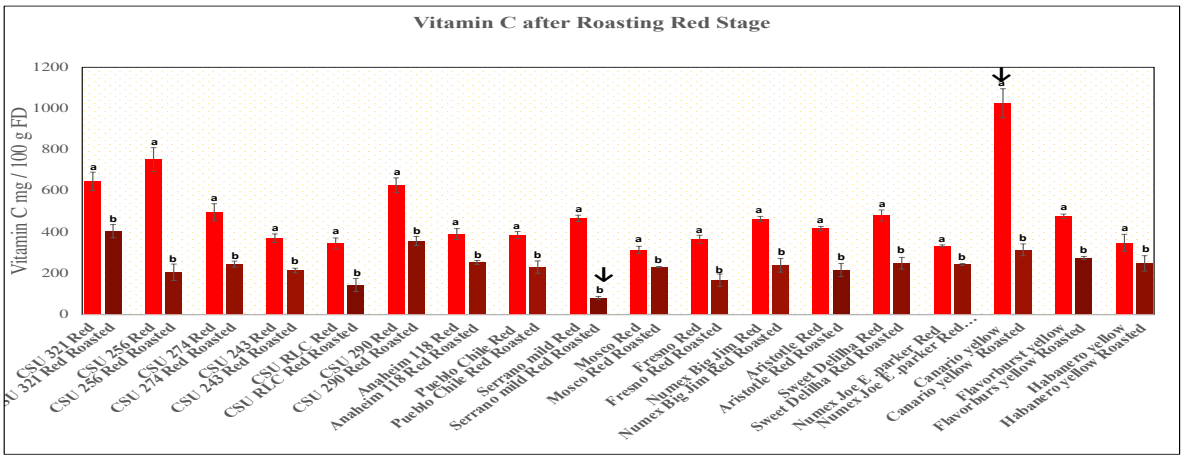


Supplemental Figure S12. Effect of roasting method on antioxidant activities of green and red pepper cultivars that grown in AVRC by ABTS assay.



Supplemental Figure S13. Levels of vitamin C of pepper cultivars that grown in AVRC in both green and red stages.





Supplemental Figure S14. Effect of roasting methods on vitamin C levels of pepper cultivars that grown in AVRC in green and red stages.

LIST OF ABBREVIATIONS

CWT:- Hundredweight or Centum Weight

ROS:- Reactive Oxygen Species

RNS:- Reactive Nitrogen Species

1-MCP:- 1-methylcyclopropene

FW:- Fresh Weight

HPLC:- High-Performance Liquid Chromatography

PDA:- Photodiode Array Detector

FCR :- Folin Ciocalteu Reagent

DPPH:- Diphenyl-1-picrylhydrazyl

ABTS :- Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)

GAE :- Gallic Acid Equivalent

QE:- Quercetin Equivalent

SHU:- Scoville Heat Units

AVRC:- Valley Research Center in Rocky Ford

SLVRC:- San Luis Valley Research Center