COMPOSITIONAL AND PHYTOCHEMICAL CHARACTERIZATION OF FOUR IMPROVED VARIETIES OF PUERTO RICO SWEET CHILI PEPPER *(CAPSICUM CHINENSE)*

By

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ABSTRACT

The pepper *Capsicum chinense,* is a key ingredient in Puerto Rican cuisine due to its flavor, aroma and in the case of local varieties, little to no pungency. Little is known about the nutritional value of this specialty pepper, locally known as 'aji dulce' or sweet chili pepper, although in general peppers are also known to be an excellent source of antioxidants such as vitamin C, β-carotene, flavonoids and phenolic compounds. The aim of this study was to determine the nutritional and physicochemical properties of the four improved varieties of Puerto Rican sweet chili pepper developed by the University of Puerto Rico Agricultural Experiment Station: 'Pasión', 'Carnaval', 'Bonanza' and 'Amanecer". The four varieties were harvested at physiological maturity. Physical, chemical and nutritional characteristics were determined. The luminosity (L*), hue angle and chroma of Pasión were distinctly different from that of the other varieties. Its green fruits had a stronger green color and its red fruits a stronger red color than other varieties. Amanecer had the longest fruit and Bonanza the largest fruit (by weight and diameter). All varieties had a high moisture content (90.57-93.28 %), low protein (0.53-0.86 %), low fat content (0.05-0.12 %), high in dietary fiber compared to other capsicum varieties (4.51-7.09 %) and were an excellent source of vitamin C (92.9-148.86 mg/100g). Pasión had the highest β-carotene content (57 mg/100g) and flavonoid content (338.51 mg QE/100g), values that were positively correlated to red color (r= 0.72 and 0.62 respectively). Red fruit of Amanecer had the highest vitamin C (148.86 mg/100g) and phenolic content (385.79 mg GAE/100g). Pungency, as measured by capsaicinoid concentration and Scoville Heat Units was very low in all varieties. Capsaicin, rather than dihydrocapsaicin, was the primary capsaicinoid present. Sweet chili pepper harvested at the red fruit stage provides higher phytochemical content and therefore might be more beneficial from a nutritional point of view than fruits harvested at the green fruit stage. Results from this study can be used by consumers to determine the potential contribution of these improved varieties to the local diet and can also be used as a basis of comparison between these varieties and new varieties of sweet chili pepper developed in the future.

RESUMEN

El pimiento, *Capsicum chinense*, es un ingrediente clave en la cocina puertorriqueña debido a su sabor, aroma y en el caso de las variedades locales, poco o ningún sabor picante. Hay poca información sobre el valor nutricional de este pimiento específico, conocido localmente como 'ají dulce'. Generalmente los pimientos son conocidos por ser una excelente fuente de antioxidantes como la vitamina C, β-caroteno, flavonoides y compuestos fenólicos. El objetivo de este estudio fue determinar las propiedades nutricionales y físico-químicas de las cuatro variedades mejoradas de ají dulce desarrolladas por la Estación Experimental Agrícola de la Universidad de Puerto Rico: Pasión, Carnaval, Bonanza y Amanecer.

Las cuatro variedades se cosecharon en su estado de madurez fisiológica. Se determinaron las características físicas, químicas y nutricionales. La luminosidad (L*), matiz y croma de Pasión fueron notoriamente diferentes de las otras variedades. Sus frutos verdes y rojos tenían colores verde y rojos más intensos que otras variedades. Amanecer tenía el fruto más largo y Bonanza el fruto más grande (en peso y diámetro). Todas las variedades tenían un alto contenido de humedad (90,57-93,28%), bajo en proteínas (0,53-0,86%), bajo contenido en grasa (0,05-0,12%), alto contenido de fibra dietética (4,51-7,09%) y eran una excelente fuente de vitamina C (92,9-148,86 mg/100 g). Pasión tuvo el mayor contenido de β-caroteno (57 mg/100 g) y de flavonoides (338,51 mg QE/100 g), valores que se correlacionaron positivamente al color rojo (r = 0,72 y 0,62 respectivamente). Los frutos rojos de Amanecer tuvieron el contenido más alto de vitamina C (148.86 mg / 100g) y fenólicos (385.79 mg GAE / 100g). Pungencia, medida por la concentración de capsaicinoides y unidades scoville, fue muy baja en todas las variedades. Capsaicina, en lugar de dihidrocapsaicina, fue el principal capsaicinoide presente. El ají dulce cosechado en la etapa de fruta roja proporciona un mayor contenido fitoquímico y por lo tanto podría ser más beneficioso desde un punto de vista nutricional que las frutas cosechadas en la etapa de fruta verde. Los resultados de este estudio pueden ser utilizados por los consumidores para determinar la contribución potencial de estas variedades mejoradas a la dieta local y también se puede utilizar como base de comparación entre estas variedades y nuevas variedades de ají dulce desarrollado en el futuro.

DEDICATION

To God, for opening doors so I could pursue higher education.

To my Mom and Dad, who have supported me with a shoulder to cry on, given me a home to live in, fed me, and picked me up from lab at midnight or at the crack of dawn if it was necessary, especially Mom for driving me to Rio Piedras two times a week for a semester.

To my big sis for being supportive and inspiring me to pursue higher education.

To my lil' sis for being so cute and huggable when I most needed it.

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A special thanks to "Team Chávez": Carolina Coronel, Fernanda Alvira, Kathina Toro and Marcela Martínez for helping me with the wonderful world of analytical chemistry and being a valuable support system through this challenging journey. Also a special thank to Magdiel, Fravelyn, Ashlie and Andrea for helping me during those long hours in lab.

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1. INTRODUCTION

Capsicum chinense is one of five domesticated species of *Capsicum* spp belonging to the Solanaceae family. It's an important part of the human diet for its flavor characteristics and uses. In Puerto Rican cuisine the sweet chili pepper, known locally as "ají dulce", is the main ingredient of a seasoning sauce, "sofrito", used to add a characteristic flavor to various typical dishes. This small red pepper is sweet and non-pungent unlike other peppers belonging to *C. chinense* which are generally pungent (Jarret, Baldwin, Perkins, Bushway, & Guthrie, 2007).

The Puerto Rican varieties (landraces) of sweet chili pepper were likely selected over generations by local farmers. According to L. Wessel-Beaver (personal communication, August 7, 2014) "in Spanish, the word '*ají'* generally refers to small pungent peppers, which can be of various *Capsicum* species. The addition of the term '*dulce',* which means sweet in Spanish, evokes the idea that '*ají dulce'* is a small, red, flavorful pepper without the strong pungency of other chili peppers of similar appearance". Sweet chili pepper is a *Capsicum* fruit that has multiple uses during different stages of maturity, but in general it is utilized as an ingredient to add pungency and flavor to food (Yogeesha & Gowda, 2003).

One of the characteristics of a pepper is its distinctive green and red color. A red pepper's distinctive color is due to the carotenoid pigments β-carotene and pro-vitamin A, which are found in the highest amounts in red peppers (Marín, Ferreres, Tomás-Barberán, & Gil, 2004). Carotenoids in the human diet have been reported to protect from cancer, stroke, cataracts, and macular degeneration among others (Mayne, 1996). Peppers

also contain ascorbic acids, flavonoids and phenolic compounds, which have redox properties attributed to the hydroxyl groups and chemical structures (Campos, Gómez, Ordoñez, & Ancona, 2013). In clinical studies antioxidant nutraceuticals (or functional foods), contributed from daily diets, inactivate the reactive oxygen species or are required as cofactors for antioxidant enzymes, playing a significant role in the prevention of various clinical conditions such as heart attack, cataractogenesis, retinal damage, cancer, asthma and much more (Lee, Koo, & Min, 2004).

Local farmers need varieties of *C. chinense* with desirable traits such as yield, quality, improved flavor, nutrition and pest resistance. This study was undertaken to determine physicochemical and nutritional characteristics of four improved varieties developed by the plant-breeding program of the Puerto Rico Agricultural Experiment Station (AES) of the University of Puerto Rico (UPR). The results of this research will contribute to the characterization of these four Puerto Rican *C. chinense* varieties in terms of their potential commercial traits and nutritional contribution to the Puerto Rican diet.

2. OBJECTIVES

Main objective: establish nutritional and physicochemical properties of four varieties of Puerto Rican sweet chili pepper.

Specific objectives:

- Determine physicochemical characteristics: Color, firmness, weight, length, diameter, pericarp thickness, pH, total acidity, and total soluble solids and reducing sugars
- Determine nutritional characteristics: proximal analysis, mineral content, total β-carotene, vitamin C, flavonoids, total phenolic compounds, and antioxidant activity.
- Determine capsaicinoid content and pungency through HPLC and sensory analysis.

3. LITERATURE REVIEW

3.1. *Capsicum chinense*

3.1.1. Puerto Rican sweet chili pepper

Peppers belong to the *Capsicum* genus. The Latin word *capsicum* comes from a Greek word (Kapto) meaning "to bite" referring to the heat sensation or pungency typical of many pepper varieties. Within this genus there are five common species: *C. annuum, C. frutescens, C. chinense, C. baccatum and C. prubescens* (Basu & De, 2003). The different varieties within the species have distinctive characteristics and physiological and chemical composition.

Sweet chili pepper, known in Puerto Rico as "ají dulce", is a type of *C. chinense*. The sweet chili pepper forms part of the daily diet because of its distinctive flavor and aroma. Not only does it contribute a desirable characteristic to local cuisine, but it potentially a good source of antioxidants such as vitamin C, flavonoids, and polyphenols, since other types of *C. chinense* have been found to have these traits (Campos et al., 2013; Meckelmann et al., 2013). However those studies were done on pungent types of *C. chinense* while Puerto Rican types are non-pungent.

3.1.2. Production value of Puerto Rican *C. chinense*:

Peppers in general are considered of great importance to the trade and local markets. Worldwide production in 2013 (latest statistical report by FAO) was 31,144,561 tons with an export value of $4,959,269 (FAOSTAT, 2013). In Puerto Rico the gross income of *C. chinense* production for 2013-2014 was 1602 tons ($2,139,000),

and the local market value per pound in 2009-2010 (latest report by the agricultural department) was of $4.01. Nevertheless during 2010 the local production of sweet chili pepper only supplied 2/3 of the local demand; the rest was imported from the Dominican Republic.

3.2. Physical parameters as quality parameters:

Physical as well as chemical parameters are commercial indicators of maturity and quality of fruit (Tadesse, Hewett, Nichols, & Fisher, 2002), and this information is crucial for farmers and processors. Fruits and vegetables are valued by their quality, which are a combination of properties including appearance, texture, flavor and nutritional value (Kader, 1983). Parameters are prioritized differently for plant breeders, market distributors and consumers. Plant breeders prioritize yield, disease resistance, harvest simplicity and shipping quality; market distributors prioritize appearance, firmness and storage life; consumers prioritize appearance, firmness, flavor and nutritional value.

Visual factors of quality can be determined by measuring size dimensions, weight, shape and form, color intensity, and defects. Texture factors can be determined by measuring fruit firmness and flavor factors can be determined through sensory analysis (Kader, 1983). Texture depends on the biomolecules in the cellular structure of cell walls. The cell wall consists of a complex structure of pectin polysacharides embedded with cellulose microfibrils and cross linking glycans, which are altered by the natural physiological transitions during ripening process such as thining of cell walls, decreased turgidity and degradation of pectin (Alberts et al., 2002; Bartz, Brecht, &

Weichmann, 2003; Jen, 1989).

3. 3. Chemical composition of *Capsicum chinense*

Peppers are considered to be a good source of essential nutrients such as vitamin C and A (Bosland & Votava, 2000). They are also a good source of carotenoids, flavonoids and mineral elements (Lee, Crosby, Pike, Yoo, & Leskovar, 2005). Peppers have a high moisture content, low protein, fat, and carbohydrate content (Table 1).

Table 1: Nutrient Data for peppers *Capsicum* spp.

	Sweet, green, raw[1]	Sweet, red, raw[2]	Hot chili, green, raw[3]	Hot chili, red, raw[4]	Jalapeño, raw[5]
Proximate Analysis					
Moisture (%)	93.89	92.21	87.74	88.02	91.69
Energy (kcal/100g)	20	31	40	40	29
Protein (%)	0.86	0.99	2	1.87	0.91
Fat (%)	0.17	0.3	0.2	0.44	0.37
Carbohydrate (%)	4.64	6.03	9.46	8.81	6.5
Dietary Fiber (%)	1.7	2.1	1.5	1.5	2.8
Sugars, total (%)	2.4	4.2	5.1	5.3	4.12
Minerals					
Ca (mg/100g)	10	7	18	14	12
Fe (mg/100g)	0.34	0.43	1.2	1.03	0.25
Mg (mg/100g)	10	12	25	23	15
P (mg/100g)	20	26	46	43	26
K (mg/100g)	175	211	340	322	248
Na (mg/100g)	3	4	7	9	3
Zn (mg/100g)	0.13	0.25	0.3	0.26	0.14
Vitamins					
Total ascorbic acid (mg/100g)	80.4	127.7	242.5	143.7	118.6

Source: USDA Nutrient Data Base, Release 28 (US Department of Agriculture, Agricultural Research Service, 2015) 1: *Capsicum annuum* ; 2: *Capsicum spp.*; 3: *Capsicum frutescens*; 4: *Capsicum frutescens*; 5: *Capsicum annuum*.

Chemical parameters are determined as quality parameters of ripe fruit; these characteristics are related to sensory characteristics such as sweetness and sourness of the fruit (Luning et al., 1994). As the fruit matures, sweetness increases because it is closely related to glucose, fructose and total sugars. Sourness also increases as the fruit matures because the citric acid and ascorbic acid of the fruit increases during ripening. Compositional changes are due to senescence of the fruit as time progresses, including changes in firmness as the polysaccharides in the cell walls break thus increasing sugar levels in the fruit (Antoniali, Leal, Magalhães, Fuziki, & Sanches, 2007). There is little information on physicochemical parameters of *C. chinense*. Table 2 summarizes the chemical parameters of *C. chinense* varieties previously studied by various authors (Campos et al., 2013; Pino et al., 2007; Reis et al., 2013). All varieties were pungent peppers.

Table 2: Chemical parameters of *Capsicum chinense* in literature cited

Reference and country of origin	(Pino et al., 2007) Yucatan, Mexico	(Reis et al., 2013) (Dry basis) Pará, Brazil	(Campos et al., 2013) Yucatan, Mexico
Soluble Solids (ºBrix)	4.6-9.1	-	-
pH	4.9-5.4	-	-
Ash (%)	0.5-1.2	-	-
Capsaicinoids (mg/g)	41.8-65.9	-	-
Capsaicin (mg/g)	-	3.40	-
Dihydrocapsaicin (mg/g)	-	0.44	-
Vitamin C (mg/100g)	-	-	187.24- 281.73

3. 4. Antioxidants

3. 4. 1. Reactive oxygen species

The main cause of aging, pathogenesis of diseases and mitochondrial degradation is the presence of reactive oxygen species (ROS) and free radicals, which are produced in the metabolic pathways of aerobic organisms. Free radicals contain an unpaired electron in atomic orbital; they are unstable and highly reactive by donating or accepting electrons (Lobo, Patil, Phatak, & Chandra, 2010). The free radicals produced in the metabolic pathways are hydroxyl radicals, oxygen singlet, hydrogen peroxide, superoxide radical, and others (Mittler, 2002). The mitochondrion consumes over 90% percent of the oxygen and is the main source of ROS and free radicals. These by-products can cause damage to DNA, protein and lipids by attacking macromolecules which cause cell damage and homo-static disruption (Ames, Shigenaga, & Hagen, 1993; Lee et al., 2004; Lobo et al., 2010). Other sources of ROS are environmental toxins, for example, those caused by smoking, exposure to X-rays, air pollutants, and industrial chemicals (Bagchi & Puri, 1998)

3. 4. 2. Importance of antioxidants:

Antioxidants can interact with free radicals preventing free radical tissue damage. Through natural metabolism the body only produces glutathione, ubiquinol and uric acid. Essential micronutrients cannot be produced and should be consumed (Lobo et al., 2010). Antioxidants are an important part of human daily intake because they can inhibit or delay oxidation (loss of electrons) thus slowing the aging process and increasing life span (Lee et al., 2004). An antioxidant deficient diet can cause oxidative stress, and an

imbalance of ROS and antioxidants, which can lead to atherosclerosis, cancer, aging and inflammatory diseases (Lobo et al., 2010). Biologically antioxidants can be enzymatic or non-enzymatic; and dietary antioxidants can be classified in two categories (Huang, Ou, & Prior, 2005). One group is "sacrificial", scavenging ROS and Reactive Nitrogen Species (RNS) to stop radical chain reactions. The other group inhibits the ROS from being formed. In a study done comparing the antioxidant properties of various *Capsicum* spp. accessions, *C. baccatum* had the highest concentrations of ascorbic acid (1.6 mg/g FW) and total phenols (1.4 mg/g FW) while *C. chinense* contained 1.2 and 1.3 mg/g FW respectively (Antonious, Kocchar, Jarret, & Snyder, 2006). In a study comparing antioxidants in *C. chinense* from various countries, including USA, Peru, Brazil, Ecuador, Mexico, Colombia, Belize and Puerto Rico, the Puerto Rican accessions had 1.25 to 1.80 mg/100g total phenols (Antonious, Lobel, Kochhar, Berke, & Jarret, 2009).

3. 4. 3. Ascorbic acid:

Ascorbic acid (AA) is a sugar acid with a furanose ring (lactone of a sugar acid) and it is an electron donor and reducing agent. AA has two biochemical functions: as an antioxidant and as an enzymatic cofactor (Rahman & Fontés, 2013). It can be synthesized by plants and most animals from glucose through an enzymatic pathway using L-gulonolactolase enzyme, not present in primates (Isherwood, Chen, & Mapson, 1954). AA is necessary in human nutrition because it is able to cure various clinical symptoms such as scurvy and it can efficiently scavenge toxic free radicals and ROS formed during cell metabolism (Arrigoni & De Tullio, 2002). The Dietary Reference Intake (DRI) or estimated average requirements for Vitamin C for adults is from 75 to 90 mg/d (Institute of Medicine, 2000). The AA content in varieties of *C. chinense* originating from various

countries ranged between 300-700 μ g/g of fresh fruit (Antonious et al., 2009). In *Capsicum spp*. the AA content increases as the fruit matures. It has been reported that among red peppers there is a 50% increase in AA compared to green peppers (Martinez, Lopez, Gonzalez-Raurich, & Alvarez, 2005) and the highest AA content in fruits occurs at 63 days after fruit set (Siddiqui et al., 2012). AA content can be affected by storage temperature and maturity stage in which it is harvested (Martinez et al., 2005).

3. 4. 4. β -carotene

β -carotene is a natural compound from the carotenoid group, a vitamin A precursor (Machlin & Bendich, 1987). As a carotenoid found in plants, β-carotene serves as accessory pigment in photosynthesis and photo protection of the plant (Mayne, 1996). Carotenoids are tetraterpenoids with conjugated polyene structure allowing the molecule to absorb light efficiently, and quench singlet oxygen and free radicals efficiently (Lee et al., 2004; Mayne, 1996). The recommended dietary allowance for pro vitamin A is 700 and 900 μg retinol activity equivalents (RAE/day) for women and men, respectively (Institute of Medicine, 2001). β-carotene contributes 12 μ g in 1 RAE, other 48 μ g are attributed to a carotene and β -cryptoxanthin. Total carotene content in previously studied *C. chinense* originating in Mexico was 1.00 to 1.26 mg/100g of sample (Campos et al., 2013).

3. 4. 5. Polyphenols

Phenolic compounds are secondary metabolites that contain an aromatic ring with at least one hydroxyl substitute (Khoddami, Wilkes, & Roberts, 2013). They function to protect plants against stress caused by environmental or biological factors such as pathogens, infections and exposure to radiation or UV(Kennedy & Wightman, 2011). Phenolic phytochemicals contain a phenolic ring and hydroxyls that function as antioxidants because they can quench free electrons (Shetty, 2004). The polyphenolic compounds are glycosided which have various subcategories, one of them being flavonoids, which contains a subgroup known as anthocyanins. Phenolics are attributed to the organoleptic properties of plant foods, bitterness and astringency are due to the interaction that phenolics have with the glycoprotein in our saliva (Dai & Mumper, 2010).

3. 4. 6. Flavonoids

Anthocyanins are a group of flavonoids that are attributed to colors in flowers, fruits and leaves, from pink to reds and violets to dark blues. Anthocyanins consist of an anthocyanidin called aglucone, as well as sugars and acyl groups (Andersen & Jordheim, 2006). There are various flavonoids found in foods, quercetin being the major flavonoid found in vegetables (Hertog, Hollman, & Katan, 1992). Anthocyainins are common water-soluble pigments that are primarily located in the vacuolar of the epidermal cells (Andersen & Jordheim, 2006). Table 3 demonstrates the phenolic compound content of *C. chinense* varieties previously studied from different countries of origin (Antonious, et al., 2009; Campos et al., 2013; Castro-Concha et al., 2014; Meckelmann et al., 2013;

Siddiqui et al., 2012; Simionato et al., 2015).

Table 3: Amount of phenolics found in previously studied *Capsicum chinense* varieties

Reference	Country of origin	Phenolic content as reported
(Campos et al., 2013)	Yucatan, Mexico	20.54-20.75 mg/100g DW
(Antonious, et al., 2009)	Mexico	<34.9mg CA/100g FW
(Siddiqui et al., 2012)	West Bengal, India	~330 mg CE/100g FW
(Castro-Concha et al., 2014)	Yucatan, Mexico	27000 mg GAE/100g FW
(Simionato et al., 2015)	Brazil	1.24, 1.74 mg GAE/100g FW
(Meckelmann et al., 2013)	Ucayali, Peru	3,690 mg GAE/100g DW

DW = Dry fruit weight
FW = Fresh fruit weight
CA = Chlorogenic Acid
CE = Catechol equivalents
GAE = Gallic acid equivalents

3. 4. 7. Antioxidant activity and antioxidant capacity assay principles:

To measure radical or oxidant scavenging capacity, antioxidant capacity assays are done. There are two main groups: hydrogen atom transfer (HAT) assays, and electron transfer (ET) assays. HAT assays quantify the antioxidant capacity by reaction kinetic curves. They consist of an azo radical initiator, molecular probe, antioxidant and reaction kinetic parameters. On the contrary ET assays measure the antioxidant capacity by measuring the reducing capacity, where an oxidant interacts with an electron from the antioxidant causing the oxidant to change color and the change in color is proportional to antioxidant concentrations (Huang et al., 2005)

3. 4. 7. 1. DPPH method

The 1-Diphenyl-2-picryl-hydrazyl (DPPH) method is based on the fact that DPPH is an N-centered stable free radical. DPPH accepts hydrogen from a donor in the extract

of the sample causing the solution to lose its deep purple color. The absorbance is read at 515 nm wavelength (Tirzitis & Bartosz, 2010). A DDPH percentage is then calculated and this value is proportional to antioxidant concentration.

3. 4. 7. 2. ORAC method

The oxygen radical absorbance capacity method (ORAC) is a method that measures antioxidant activity combining both the inhibition time and degree of inhibition. Results are expressed in ORAC units, an indication of the protection produced by antioxidants. This method uses 2,2'-azobis (2-amidinopropane) dihydrocloride (AAPH) as a peroxyl radical generator, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as a standard (Cao, Alessio, & Cutler, 1993) and fluorescein (3',6'-dihydroxyspiro (isobenzofuran-1[3H],9'[9H]-xanthen)-3-one) (FL) as the fluorescent probe to be used with the micro plate fluorescent reader method (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). The ORAC fluorescein method provides a measurement of hydrophilic chain-breaking antioxidant capacity against the peroxyl radical, without being affected by exposure to the excitation light from the fluorescent reader. As the FL is consumed the FL intensity decreases. When an antioxidant is present the FL decay is inhibited or delayed. The data is calculated as the area under the curve (AUC), in which the Trolox equivalents can be calculated with a Trolox standard curve (Huang et al., 2005).

3. 4. 8. Antioxidant analysis principles:

3. 4. 8. 1. Ascorbic acid analysis:

The 2,6-Dicholoroindophenol Titrimetric Method (AOAC 967.21) is an oxidation of L-ascorbic acid to L-dehydroascorbic acid by the redox indicator dye 2,6-dichloroindophenol (DCIP). AA is extracted from the sample with metaphosphoric acid due to the fact that the vitamin is susceptible to oxidative deterioration and needs to be performed in low pH (Pegg, Landen, & Eitenmiller, 2010). The indicator dye will have a rose-pink end point, the excess unreduced dye after the redox reaction (Figure 1)

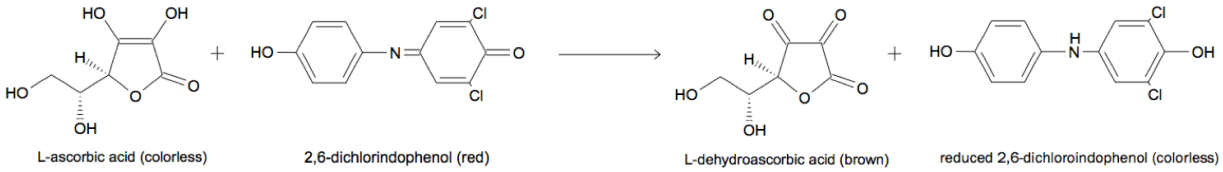


Figure 1: Chemical reaction of L-ascorbic acid and DCIP (Pegg et al., 2010).

3. 5. Capsaicinoids:

3. 5. 1. Pungency

An important characteristic of peppers in food is the flavor they attribute into local cuisine. Flavor is the combination of odor, taste sensation, texture, appearance and other psychological factors; chemical dependent flavor contributors are taste sensation (pungency) and odor (Kulka, 1967). Pungent compounds are derivatives of o-methoxyphenol (Figure 2); the main derivatives found in peppers are capsaicin and dihydrocapsaicin (Figure 3).

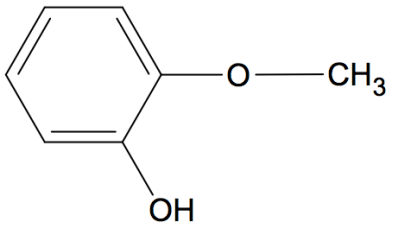
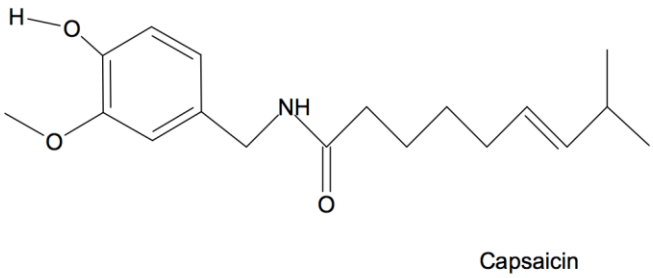


Figure 2: o-methoxyphenol Structure (Kulka, 1967)



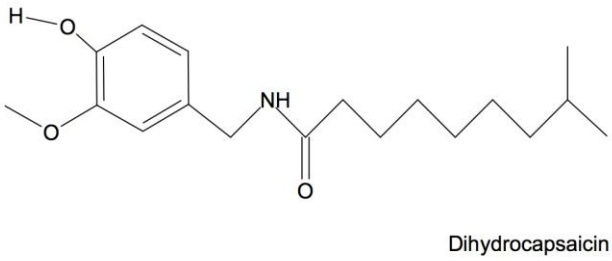


Figure 3: Capsaicin and dihydrocapsaicin structure (Kulka, 1967)

Pungency in *Capsicum spp.* fruits is attributed to compounds called capsaicinoids, which are alkaloids made up of a vanilloid, amide and a hydrophobic side chain, and are only found in peppers (Canto-Flick et al., 2008; Zewdie & Bosland, 2000). Almost all (90%) of pungency is attributed to the capsaicin (trans-8mehtyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-N-vanillylnonamide) which are unique to the genus *Capsicum* (Laskaridou-Monnerville, 1999).

3. 5. 2. Determining pungency

In 1912 Wilbur Scoville developed a sensory method that measures pungency using Scoville heat units (SHU). The method involves adding in defined proportions of a solution of capsaicin with alcohol to sweetened water until a light pungency is perceptible on the tongue (Scoville, 1912). Methods to determine capsaicinoid content have become more analytical with time. The most reliable and convenient method is the High Performance Liquid Chromatography (HPLC) method (Collins, Wasmund, & Bosland, 1995). Once the individual capsaicinoid contents are calculated with chromatographic data, the pungency can be calculated by multiplying the value of capsaicinoid content with the threshold pungency (Batchelor & Jones, 2000; Todd, Bensinger, & Biftu, 1977).

4. METHODOLOGY

4. 1. Plant Materials

Four cultivars, ‘Amanecer’, ‘Pasión’, ‘Carnaval’, and ‘Bonanza’ (Figure 4) of sweet chili pepper, *C. chinense*, developed by the plant-breeding program of the Puerto Rico Agricultural Experiment Station of the University of Puerto Rico (AES-UPRM) were studied. Fruits used in the study were harvested from field plantings at Lajas, Isabela, Mayaguez and Juana Diaz during August 2014 and May 2016. For the measure of all parameters, fruits were harvested at the commercially mature stage. Commercially mature stage is defined as the point during plant phenology where fruits have reached their full weight and size (fully developed). At each harvest, both green and red fruits were selected at random and taken to the Food Science and Technology program facilities at UPRM. The samples were washed with ambient temperature filtered water using a spray nozzle to remove all organic material. The fruits were drained to remove excess water and placed on a dry paper towel to air-dry completely. Fruit calyxes were removed before processing.

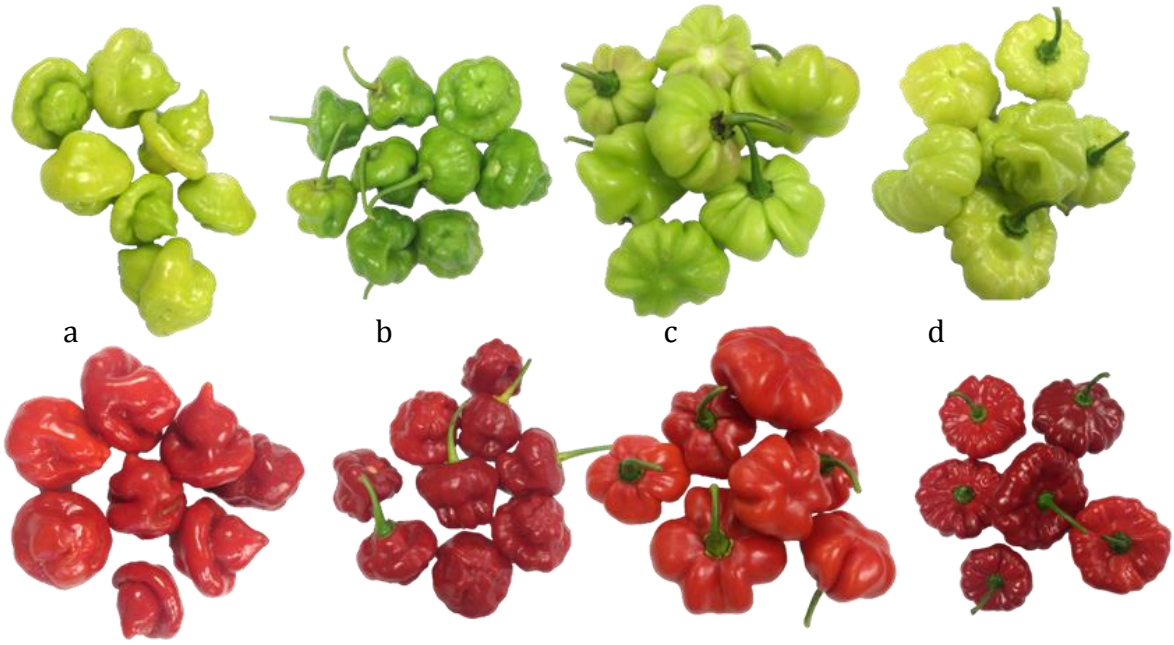


Figure 4: Four varieties of *Capsicum chinense* (a) Amanecer, (b) Pasión, (c) Carnaval, (d) Bonanza

4. 2. Physical parameters

Fruit samples were collected at each of five harvest dates (replicates). At each harvest, color and firmness was measured separately on 15 green and 15 red fruit with stems removed. All other physical parameters were measured on a mixed sample (red and green) of 15 fruit. Color was measured on both red and green fruit as L*, a* and b* with a colorimeter (Color Flex EZ, HunterLab, Reston, VA, USA) calibrated with white and black porcelain tiles. Each whole fruit was placed on the colorimeter port and covered with a black aluminum cup to avoid ambient light from affecting the sample measurement. Value of a* and b* were used to calculate hue angle (a measure of type of color) and chroma (a measure of color purity or saturation) using equations 1 and 2 (Mcguire, 1992).

Equation 1: $h^o = \tan^{-1} {}^{b^*}/_{a^*}$

Equation 2: $C* = \sqrt{a^{*2} + b^{*2}}$

If a>0 and b>0 then $h^o = \theta$ If a<0 and b≥0 then $h^o = 180^o + \theta$

If a<0 and b<0 then $h^o = 180^o + \theta$ If a>0 and b<0 then $h^o = 360^o + \theta$

Fruit firmness was determined with a texturometer (Texture Analyzer TA-XT2, Stable Micro systems, Godalming, England) with a 2 mm needle probe. The peak force was converted to newtons (N) to determine the force necessary to penetrate the pepper's pericarp.

Fruit length and diameter was measured with a digital caliper (Absolute, Digimatic caliper, Mitutoyo Corporation, Japan). Fruit weight was determined using an analytical balance (ML204/03, Mettler Toledo, Greifensee, Switzerland). Using a kitchen knife, fruit were cut and the placenta (ribs) and seeds were removed. The pericarp (seeds and placenta removed) and seeds were weighed separately, and the thickness of the pericarp wall was measured.

4. 3. Chemical Parameters

At each of the five harvest dates, the stems and seeds from 15 fruits (mixture of red and green fruits) were removed, and samples were homogenized in a food processor (Mini-prep plus 24 oz processor, Cuisinart, East Windsor, NJ, USA) until a paste-like consistency was achieved. The homogenization was done at short intervals (15-20s) to

avoid heating of the sample. Official methods described in the AOAC (Horwitz, 2003) were used to evaluate pH, titratable acidity and to carry out the proximal analysis.

Measurements of pH were done using the AOAC 981.2 method using a pH-meter (SympHony, SB70P VWR, Cornelius, OR, USA), with a calibrated electrode (AR15, Fisher Scientific, USA) and using buffer solutions with known pH values of 4.00, 7.00 and 10.00 (Orion application Solution, Thermo Fisher Scientific, MA, USA). The electrode was directly immersed into the sample paste to obtain the pH measurements. Titratable acidity was determined using the AOAC 942.15 methodology. The titration was done using NaOH 0.10N to reach a pH of 8.20. The results were expressed in citric acid percent, the major acid present in *C. chinense* (Nuñez-Ramirez, Gonzalez-Mendoza, Grimaldo-Juarez, & Diaz, 2011).

Total soluble solids expressed in ^{o}Brix were determined using a digital refractometer (Pal-1, Atago, Tokyo, Japan). A drop of sample with no solid particulates was placed on the port to be measured. The ^{o}Brix represents the sugar content in the sample; one-degree brix is equivalent to 1 gram of sucrose in 100 g of solution, percentage by mass.

A proximate analysis was conducted. Ash content was measured following AOAC 923.03, protein content was determined using AOAC 991.20 with a 6.25 conversion factor, crude fat was measured using AOCS Am 5-04, and crude fiber determined using AOCS Ba 6a-05.Carbohydrates were determined by difference.

Minerals were determined using a Microwave Assisted Acid digestion method for plant tissue (standardized method by the USDA Tropical Agricultural Station, Mayaguez, Puerto Rico). The samples and standard (peach leaf, Standard Reference Material 1547,

National Institute of Standards and Technology, Gaithersburg, MD, USA) were dried in an oven (Lindbergh Blue M, Thermo Scientific, USA) for 24 hours at 70^{o}C then placed in a desiccator until cooled.

Once the samples were at room temperature, 0.1750 grams (g) of sample was weighed into a Teflon® tube, and 2 ml of HNO_3 and 3 ml of H_2O were added to the sample. The samples were placed in the Microwave Reaction System (Multiwave 3000, Anton Paar, Gaz, Austria) for 45 minutes (850 power, 15 min ramp and 30 min hold with 20 min cool down period). Once the digestion process was completed and the samples were at room temperature the Teflon® tube was removed from the microwave and transferred to a fume hood were the solution was transferred to a 15 ml centrifuge tube, rinsed with demineralized water three times and filtered through Whatmam No. 541 filter paper to complete the 14 ml mark line.

Table 4: Mixed calibration standard units and concentrations of minerals analyzed.

	Calibration Unit: ppm*				
Analyte	Std Mix 1	Std Mix 2	Std Mix 3	Std Mix 4	Std Mix 5
P	2	5	10	15	20
K	40	100	200	300	400
Ca	30	75	150	225	300
Mg	6	15	30	45	60
Fe	0.5	1.25	2.50	3.75	5
Mn	0.2	0.5	1	1.5	2
Zn	0.1	0.25	0.5	0.75	1
B	0.1	0.25	0.5	0.75	1
Mo	0.1	0.25	0.5	0.75	1
Al	0.4	1	2	3	4
Cu	0.1	0.25	0.5	0.75	1
Na	0.2	0.5	1	1.5	2
S	0.4	1	2	3	4

*ppm=mg/L

The samples were read with an inductively coupled plasma optical emission spectroscopy (ICP-OES Optima 7300 DV, PerkinElmer, Waltham, MA, USA) using a mixed calibration curve of the standard solutions of P, K, Ca, Mg, Fe, Mn, Zn, B, Mo, Al, Cu, Na, S (SPEX CertiPrep, Metuchen, NJ, USA) (Table 4).

4. 4. Antioxidants

A sample of 15 fruits (mixture of green and red) from each of the 5 harvest dates was used to measure antioxidants with the exception of β-carotene where separate samples of 15 green and 15 red fruits were used.

4. 4. 1. Ascorbic acid content

Ascorbic Acid (AA) content was determined using the 2,6-dichloroindophenol titrimetric method (AOAC 967.21). The extract was prepared by homogenizing 4 grams of fresh homogenized pericarp sample with 10 ml of metaphosphoric acid solution (15 mg HPO_4 and 40 ml $C_2H_4O_2$ diluted to 500ml) for 2 min in a food processor then filtering with a Buchner funnel containing a Whatman No. 4 paper under suction. The filtrate was diluted with water to 50 ml. A total of 2 ml of the extract was added to a 50 ml Erlenmeyer flask with 5 ml of metaphosphoric acid solution and titrated with indophenol solution previously standardized with ascorbic acid standard solution. The results were expressed in mg of AA/mL of extract then converted to mg/g of sample.

To determine if AA content increase as fruit changed color from green to red, 15 green and 15 red fruits (with seeds and placenta removed) were analyzed. The percentage of reducing sugars was determined using the Lane Eynon method (AOAC 923.09) using the following equation:

Equation 3:

$$Reducing\ Sugar(\%) = \left(\frac{RS \times 50ml}{V_{tot} \times G \times 1000}\right) \times 100$$

In which

RS= reducing sugars (mg) required to react with 10 ml of Soxhlet solution (from Table 930.33 in appendix C of the AOAC)

V_{tot}= total volume of sample used in titration

G= sample (g)

4. 4. 2. β -carotene content

In order to later compare β-carotene measurements with color parameters, color was measured on both green and red whole fruits as previously described. Fruits were then cut, seeds were removed and the pericarp was homogenized using a food processor. Color measurements were taken on the paste samples. β-carotene content was measured using the Antonious (2009) method with minor modifications. A 7.5 g sample of fresh homogenized pericarp was weighed into a centrifuge vial covered with aluminum foil to avoid β-carotene degradation by light. A total of 25 ml of acetone was added to the vials. The sample was homogenized using a Stand Dispersion Unit (Polytron PT 2500 E, Kinematica, Luzernerstrasse, Switzerland) at 18 thousand rpm for 2 min. The sample was then centrifuged at 4500 rpm for 5 min. The supernatant was transferred to an amber vial. The pellet was extracted two more times with 25 ml of acetone. The three supernatants were mixed and filtered in a Buchner funnel with suction and Wathman No. 1 filter. The filtered extract was transferred to a separatory funnel containing 12.5 ml of NaCl 4% and

25 ml of petroleum ether, and covered in aluminum foil. The mixture was shaken for 3 min with constant pressure release and let to rest for 2-3 min. The bottom aqueous solution was discarded and the top petroleum layer volume was measured. A portion of this extract was diluted in a volumetric flask to fit in the standard curve and spectrophotometer range. The sample was read at 450 nm and the absorbance was interpolated with a β-carotene (Sigma-Aldrich 97% purity) standard curve to obtain β-carotene ppm (mg/L) in the extract.

Due to a possible underestimation using the above method, a second method was used to corroborate the results (Nagata & Yamashita, 1992). A 1 g of sample was homogenized in 20 ml of acetone: hexane solution (40:60 v/v) using a Stand Dispersion Unit at 18 thousand rpm for 2 min. The extract was read at 663, 645, 505 and 453 nm. The combined absorbance was used in equation 3 to determine mg/100g of β-carotene. A standard curve was prepared with a β-carotene standard to validate the equation.

Equation 4:

$$\beta - \text{carotene}(mg/100ml) = 0.216(A_{663}) - 1.22(A_{645}) - 0.304(A_{505}) + 0.452(A_{453})$$

4. 4. 3. Antioxidant activity

4. 4. 3. 1. Extract preparation

For total phenolics, antioxidant activity (DPPH method), antioxidant capacity (ORAC method), and flavonoid content, samples were divided into two groups, whole fruit and pericarp, and homogenized using a food processor. The homogenized sample

was frozen in a blast freezer -80ºC for 24 h and then freeze-dried for 48 h in freeze dryer (Micro Modulyo 115, Thermo Electron Corporation, Waltham, USA) with 300 ml freeze-drying flasks attached to an 8-port column. The samples were weighed before and after drying to determine average percentage moisture of each variety (whole fruit and pericarp). The dry samples were stored in vacuum-sealed bags (3400 series, Food Saver, Boca Raton, FL, USA) and stored at -18 ºC until extract preparation.

The extract preparation was a modification of the methanol extraction by Campos et al. (2013) for *C. chinense* analysis. A 0.125 g lyophilized sample was added to a 2.5 ml methanol aqueous solution at 80% (v/v) while stirring for 3 h at room temperature. The suspension was centrifuged at 3000 rpm for 20 min and the supernatant was separated and transferred to an amber vial and stored at 4 ºC. A second extraction was done and the two supernatants were mixed and stored in an amber vial at 4 ºC for no more than 24 h or until analysis (whichever came first).

Fresh tissue samples were prepared with absolute ethanol (Siddiqui et al., 2012) to compare antioxidant activity in green and red fruit with minor modifications. A sample of 0.5 g of fresh tissue (either whole fruit with seed and placenta, or pericarp, seed, and placenta) was weighed into a 15 ml centrifuge tube and a small magnetic stirrer was added. The fresh sample was extracted with 5 ml of absolute ethanol at 21-22 ºC for 2 h on a hot plate with stirrer at 1150 rpm (PC 420D, Corning, New York, USA). The extract was centrifuged for 5 min at 3000 rpm and the supernatant was transferred to vial and stored at 4 ºC. The residue was extracted twice and centrifuged, all supernatants were added to the last extract, mixed and centrifuged.

4. 4. 3. 2. Total phenolics

The total phenolic content was determined using a micro scale protocol performed in a 2.5 ml disposable cuvette (Waterhouse, 2001). A 20 μl sample of extract or standard or blank, 1.58 ml of water, and 100 μl of the Folin-Ciocalteu reagent (2N) were mixed thoroughly and incubated for 1 to 8 min 300 μl of sodium carbonate solution was mixed into the cuvette mixture and incubated for 2 h at room temperature and covered to avoid direct light to the sample. The samples absorbance was measured at 765 nm on a UV visible-spectrophotometer (UV-3100PC UV-Vis, VWR International, Radnor, PA, USA) and the results were expressed in Gallic Acid Equivalents in micrograms per gram of fresh fruit weight (FW) (μg GAE /g FW) using a Gallic acid standard curve.

A micro-scale analysis using a microplate reader (Ultramark microplate imaging system, Bio-Rad, California, USA) was done by adding 2 μl of sample/blank /standard, 118 μl of water and 40 μl Folin-Ciocalteu reagent (0.4 N) into the microplate wells. After 3 min, 30 μl of sodium carbonate solution was added and the sample was incubated at 37 ^{o}C for 1 h and then read at 750 nm on a microplate reader.

4. 4. 3. 3. Flavonoid content

The total flavonoid content was determined with the aluminum chloride method (Mihai, Mărghitaş, Bobiş, Dezmirean, & Tămaş, 2010; Vera-Guzmán, Chávez-Servia, Carrillo-Rodríguez, & G. López, 2011) in micro-scale to fit into a 2.5 ml disposable cuvette. A 250 μl aliquote of extract was mixed with 750 μl of ethanol 95%, 50 μl of 10% aluminum chloride hexahydrate, 50 μl of potassium acetate 1 M and 1.4 ml of

deionized water. The mixture was homogenized and incubated at room temperature for 40 min. The reaction was measured at 415 nm on the UV visible-spectrophotometer. The results were determined using a calibration curve using a quercetin standard (>95%, Sigma-Aldrich, MO, USA) and expressed as quercetin equivalents (QE) in milligrams over 100 g of fresh weight.

For fresh fruit tissue samples extracted with absolute ethanol, the total flavonoid content was determined using the aluminum chloride method (Siddiqui et al., 2012). A 1 ml sample of extract was added to a 10 ml volumetric flask containing 4 ml of deionized water, 0.3 ml of 5% $NaNO_2$ and 0.3 ml of 10% $AlCl_3$. After 6 min at room temperature, 2 ml of 1 M NaOH was added and diluted to 10 ml with deionized water. The flask was vortexed and the solution was measured at 510 nm in the UV-Vis spectrophotometer.

4. 4. 3. 4. Antioxidant activity (DPPH Method)

A DPPH solution in 80% aqueous methanol was prepared to have an initial absorbance of 1.00-1.10 (in 517 nm wavelength) (Kim, Lee, Lee, & Lee, 2002; Kuskoski, Asuero, Troncoso, Mancini-Filho, & Fett, 2005). In a 4 ml cuvette, 2.9 ml of the methanolic DPPH solution was added and the initial absorbance was measured. Immediately 0.1 ml of extract was added, mixed thoroughly and let to stand in a dark room at room temperature for 30 min. The absorbance was determined at 517 nm and the results were expressed in Trolox Equivalents (TEAC μ M/g fresh samples) using a Trolox calibration curve through linear regression.

4. 4. 3. 5. Oxygen Radical Absorbance Capacity, ORAC

The antioxidant capacity was determined using the ORAC method (Ou, Hampsch-woodill, & Prior, 2001) based on the protection of fluorescein by the sample extract against a free radical 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH), combining the degree of protection and time. The samples were prepared using 80% methanol. The sample was then diluted adding 1 μl to 499 μl (dilution factor=500) of 80% methanol to fit in the standard curve range. A phosphate buffer (7.4 pH) was prepared combining 75 ml of 0.75 mol/L potassium phosphate (K_2HPO_4) and 25 ml of 0.75 mol/L sodium phosphate (NaH_2PO_4). This buffer was used to prepare the fluorescein, AAPH and Trolox solutions. The fluorescein solution was prepared by diluting a previously prepared 440 mg/L stock solution to 93.54 nmol/L on the day of analysis. The AAPH solution (60 g/L) was prepared on the day of analysis and kept at 0^{o}C. A Trolox stock solution was prepared by dissolving 0.0050 g of Trolox in 50 ml of phosphate buffer then diluted to concentrations ranging from 6.25 to 100 μM (Table 5). A 96 well black plate with clear bottom polystyrene plate (Costar®, Corning, NY, USA) was used. In each well, 50 μl of diluted sample, standard or blank was added followed by150 μl of fluorescein solution. The plate was inserted into the microplate reader set for a 10 sec shake and incubation at 37^{o}C for 15 min. After the samples and fluorescein were incubated, 50 μl of AAPH was added immediately. Reading was taken every minute for 60 min at 37^{o}C at an excitation of 493 nm and emission of 515 nm (fmax Type 374, Molecular Devices®, Sunnyvale, CA, USA). The area under the curve (AUC) was calculated for blank, standard and samples (See Appendix 9.1) and the Net AUC was determined using the Blank AUC (See Appendix 9.1.4.). The ORAC μM TE/L of the

samples was determined by the standard curve (6.25-100 μM), then the results were converted to mmol/g using the dilution factor, sample weight and solvent volume.

Table 5: Trolox standard curve dilutions

Concentration (μM)	(μl)	Phosphate Buffer (μl)
100	250 μl stock solution	700
50	500 μl of 100 μM solution	500
25	500 μl of 50 μM solution	500
12.5	500 μl of 25 μM solution	500
6.25	500 μl of 12.5 μM solution	500
Blank	0 μl	1000

4. 4. 4. Pungency

4. 4. 4. 1. Preparation of capsaicin and dihydrocapsaicin stock solutions

A total of 0.515 mg of dihydrocapsaicin analytical standard (>97.0% analytical standard Sigma-Aldrich, St. Louis, MO, USA) was weighed into a 5 ml volumetric flask and taken to volume with 100% methanol HPLC grade (Fisher Scientific Company, USA) resulting in a 100 ppm solution of dihydrocapsaicin. A total of 0.505 mg of capsaicin analytical standard (>99.0% analytical standard Sigma-Aldrich, St. Louis, MO, USA) was weighed into a 5 ml volumetric flask and taken to volume with 100% methanol HPLC grade (Fisher Scientific Company, USA) resulting in a 100 ppm solution

of capsaicin. A total of 2.5 ml of each solution was added to a flask resulting in a 50:50 ppm dihydrocapsaicin: capsaicin standard stock solution.

4. 4. 4. 2. Determination of capsaicinoids

A standard curve was prepared by using the 50:50 stock solution and diluting it until reaching three standard solutions with the following concentrations: 10 ppm, 1 ppm and 0.1 ppm. The standard solutions were injected into a Waters Novapack C18 4 μm 3.9x100 mm column (Waters, Milford, MA, USA) using the volumes in Table 6 to achieve the calibration curve. The stationary phase was a Waters Novapack C18 4 μm 3.9x100 mm column. The mobile phase was a gradient phase with 2 solvents: Solvent A was an aqueous solution of 10% methanol and Solvent B was 100% methanol. The run time was 20 min, in which the first 10 min the solvent ratio was 43% A and 57% B. the final10 min the solvent ratio was 32% A and 68% B. A 50 μl aliquot of sample/standard passed through a Waters Fluorescent detector (470 scanning fluorescent detector, Waters, Milford, MA, USA) and was read at 280 nm excitation and 338 nm emissions with a retention time of 10 min.

The concentration was determined using an external standard (capsaicin and dihydrocapsaicin HPLC analytical standards). A calibration curve was expressed as peak areas of the standard versus concentration of the standard in ppm for each standard concentration. Based on the standard curve's linear regression equations (Figure 12, Appendix 9.1.5), capsaicin concentration (C ppm) and dihydrocapsaicin concentrations (D ppm) were determined using equations 5 and 6.

Equation 5: $C\ ppm = (peak\ area\ of\ sample - 7678.5)/\ 825281$

Equation 6: $D\ ppm = (peak\ area\ of\ sample - 5632.3)/\ 848152$

Table 6: Preparation of standard solutions for determining the capsaicinoid calibration curve.

	Initial Capsaicinoid concentration capsaicin:dihydrocapsaicin (ppm*)	Injected volume (μ l)	Final concentration (ppm)*
Standard 1		50	10:10
Standard 2	10:10	25	5:5
Standard 3		12.5	2.5:2.5
Standard 4		50	1:1
Standard 5	1:1	25	0.5:0.5
Standard 6		12.5	0.25:0.25
Standard 7		50	0.1:0.1
Standard 8	0.1:0.1	25	0.05: 0.05
Standard 9		12.5	0.01:0.01

*ppm=mg/L

4. 4. 4. 3. Verification of extraction methodology

The extraction method was verified using Jalapeño pepper samples (from L. Beaver), spiked Jalapeño samples and unspiked samples. Jalapeño peppers are known to be pungent. Freeze dried jalapeño sample was prepared the same way the sweet chili pepper samples were prepared. A capsaicinoid solution of 5 ppm was prepared in 5 ml of

acetonitrile. Jalapeño samples were spiked with 1.5 ml of a 5 ppm capsaicinoid solution in acetonitrile and 3.5 ml acetonitrile resulting in a 1.5 ppm spiked solution. The recovery percent (R%) was determined using the following Equation:

Equation 7: $$R\% = \frac{(Spiked\ sample\ result - unspiked\ sample\ result)}{known\ spike\ added\ concentration} x100\%$$

4. 4. 4. 4. Scoville heat unit

Scoville Heat Units (SHU) measure the total 'heat' or pungency of a sample based on the combined effects of capsaicin and dihydrocapsaicin and any other capsaicinoid. SHU of each sample were determined using equation 8, in which the concentration of each sample was converted to SHU using a conversion factor (16.1) based on the SHU of pure capsaicin.

Equation 8: $$SHU = \left(\frac{[PAC + (0.82)(PAD)](ppm\ standard)(ml\ acetonitrile)}{(capsaicin\ peak\ area\ of\ standard)(g\ sample)}\right) \times 16.1$$

Where

PAC= peak area under the curve of capsaicin

PAD= peak area under the curve of dihydrocapsaicin

4. 4. 6. Sensory analysis

The sensory evaluation of pungency was done using the standard test method for sensory evaluation of low heat chilies (E1395-90) (ASTM, 2011) (Appendix 9.3). Thirteen volunteer panelists were trained to be able to identify various concentrations of

capsaicin in a solution. After the final training session, the 8 panelists that marked the correct answer were selected to do the final sensory analysis of the variety samples.

4. 5. Statistical analysis

All samples were analyzed in triplicates (three extracts, three samples, three results) with exception of ORAC, which was done in quadruplicate due to the sensitivity of the analysis. Triplicate or quadruplicate data was averaged before carrying out the analysis of variance based on a completely randomized design. The statistical analysis was done using the Infostat software (Di Rienzo et al., 2014). Means were compared using Fisher's least significant difference at 0.05 probability level. The correlations between antioxidant properties and color parameters were done using the Pearson correlation coefficient (r).

5. RESULTS AND DISCUSSION

5. 1. Color parameters

Within each variety, L* (luminosity measured from 0 being black [no color] to 100 being white) decreased as fruit color state changed from green to red (Table 7). L* in green fruit averaged 54.2, decreasing to an average of 38.2 in red fruit. The luminosity of green fruits of Pasión was considerably lower than that of the other three varieties, and even lower than the red fruit of Carnaval. As the fruit color stage changed from green to red, the hue angle (an indication of type of color) decreased from yellow-green (99.5-107.6º) to red-orange (34.0-37.6º) (Table 7). At both the green and red fruit color state, Pasión was distinct from the other varieties. Compared to the other varieties, hue angle for Pasión was greater (more green) at the red fruit state and lower (more red) at the red fruit stage.

The color saturation or purity was determined using the chroma value. Colors with a higher chroma are more vivid while colors with a lower chroma are duller. Chroma values for Pasión were again distinct from those of the other varieties. Pasión had the lowest chroma value for both red and green fruit indicating that both its green and red stage colors were less saturated or vivid. The distinct green and red color of Pasión could easily be seen with the naked eye and was one of the reasons this variety was selected (L. Beaver, personal communication).

Table 7: Means of surface color parameters (L*, hue angle and chroma) of green and red fruit of four improved varieties of *Capsicum chinense.*

Variety	Fruit color stage	L*	Hue angle (^{o})	Chroma
Amanecer	Green	57.9^{e}	100.5^{c}	47.2^{b}
Pasión	Green	40.0^{c}	107.6^{d}	37.0^{a}
Carnaval	Green	59.5^{e}	101.4^{c}	48.6^{bc}
Bonanza	Green	59.4^{e}	99.5^{c}	45.6^{b}
Amanecer	Red	36.8^{ab}	37.6^{b}	52.7^{d}
Pasión	Red	33.9^{a}	34.0^{a}	47.7^{b}
Carnaval	Red	43.5^{d}	37.6^{b}	52.9^{d}
Bonanza	Red	38.7^{bc}	37.1^{b}	51.4^{cd}
F-LSD		3.2	2.8	3.3

Means in the same column with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test.
F-LSD = Fisher's least significant difference at the 0.05 probability level.

5. 2. Firmness

There were no differences in firmness between red and green fruit of the same variety (Table 8). The least firm fruit was Pasión in both red and green color stages (5.92 and 5.75 N respectively). These results are in contrast to a study done on *C. annuum* (Lurie, Shapiro, & Ben-Yehoshua, 1986), where they concluded that water soluble pectin increases in peppers as the fruit changes from green to red thus reducing the firmness. Another study done on sweet peppers (Tadesse et al., 2002), attributed increasing fruit firmness to changes in pericarp thickness as the fruit matures. In this study, pericarp thickness was measured on a sample of both green and red fruits, not each fruit color stage separately. Therefore it is unknown if there was a relationship between fruit firmness and possible changes in pericarp thickness as fruits changed from green to red in color.

Table 8: Firmness of pericarp of green and red fruit of four varieties of *Capsicum chinense*

Variety	Fruit color stage	Firmness (N)
Amanecer	Green	8.12[c]
Pasión	Green	5.75[a]
Carnaval	Green	8.04[bc]
Bonanza	Green	7.31[b]
Amanecer	Red	8.06[c]
Pasión	Red	5.92[a]
Carnaval	Red	8.11[c]
Bonanza	Red	7.79[bc]
F-LSD		0.73

Means with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test.
F-LSD = Fisher's least significant difference at the 0.05 probability level.

5. 3. Physical parameters

Fruit weight ranged between 7.83-17.14 g (Table 9). Carnaval had the heaviest fruit. The pericarp weight ranged between 6.57-17.09 g, and Carnaval again had the highest weight. There were no significant differences among varieties for the weight of placenta and seeds. On average, 90.3% of a fruits weight was from its pericarp and 9.7% from its placenta and seeds combined. Fruit diameter ranged between 37.1-43.3 mm and length ranged between 23.4-36.9 mm. Amanecer had the longest fruit, and there were no significant differences between the other three varieties. Pericarp thickness ranged between 2.0-4.2 mm. Carnaval had the thickest pericarp among the varieties.

Table 9: Means of fruit weight (whole fruit, pericarp and placenta + seeds) and size among four varieties of *Capsicum chinese*.

	Weight (g)			Percentage of whole fruit weight		Size (mm)		
	Whole fruit	Pericarp	Placenta	Pericarp	Placenta and seeds	Fruit diameter	Fruit length	Fruit pericarp
Amanecer	12.4^{a}	10.83^{ab}	1.52^{a}	88.75^{ab}	11.25^{ab}	39.56^{ac}	36.88^{a}	2.73^{ab}
Pasión	7.83^{b}	6.57^{a}	1.24^{a}	88.05^{a}	11.95^{b}	37.12^{a}	24.34^{b}	1.97^{a}
Carnaval	17.14^{c}	17.09^{c}	1.26^{a}	92.62^{b}	7.38^{a}	43.3^{b}	23.38^{b}	4.21^{c}
Bonanza	12.7^{a}	11.34^{b}	1.09^{a}	91.6^{ab}	8.4^{ab}	42.16^{bc}	24.53^{b}	2.91^{b}
F-LSD	3.29	4.34	0.50	4.57	4.57	2.69	3.22	1.75

Means in the same column with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test.
F-LSD= Fishers least significant difference at the 0.05 probability level.

5. 4. Chemical parameters

The percentage of acidity (percentage of citric acid) ranged from 0.30 to 0.41 %, Bonanza fruits were significantly more acidic than other varieties (Table 10). The pH of fruit paste ranged between 4.84 and 5.11 making it an acidic fruit. The pH of Bonanza was significantly lower than Carnaval but not different from other varieties. The soluble solids or ^{o}Brix ranged from 5.63 and 6.42 where Bonanza was significantly higher than the other varieties.

Table 10: Means of chemical parameters of four varieties of *Capsicum chinense*

	Acidity (%)	pH	Soluble Solids (^{o}Brix)
Amanecer	0.35^{a}	4.84^{a}	6.19^{a}
Pasión	0.33^{ab}	4.87^{a}	6.31^{a}
Carnaval	0.30^{b}	5.11^{b}	5.63^{b}
Bonanza	0.41^{c}	4.86^{a}	6.42^{a}
F-LSD	0.03	0.14	0.50

Means in the same column with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test.
F-LSD= Fishers least significant difference at the 0.05 probability level.

The physicochemical parameters of *Capsicum* vary between varieties and species. The four varieties analyzed had a low pH, percentage acidity and soluble solids (Table 10). The pH values were comparable to previously studied *C. chinense* varieties that have a pH of 4.55-5.79 but total soluble solids of these varieties were on the low range ofthe 6.50-12.41% that has been previously reported in *C. chinense* from Brazil (Rêgo, Rêgo, Matos, & Barbosa, 2011). Values were comparable to that reported for *C.annuum* and *C. frutences* from the

USDA database (USDA, 2016) (2.4-5.3% Table 1). These chemical parameters vary greatly among fruit accessions due to maturity.

5. 5. Proximal analysis and micro nutrients

The proximal analysis results of the four Puerto Rican varieties are comparable to *Capsicum* spp (*annuum*, *frutences*, spp.) in the USDA nutrient database (USDA, 2016) (Table 1). The four genotypes of *C. chinense* presented an average moisture percentage of 92.31%, dietary fiber of 5.36%, protein of 0.66%, 0.08% fat content and 0.57% ash content in the pericarp of the fruit (Table 11). With the exception of iron (Fe) and sodium (Na) there were differences among the four varieties for all components of the proximate analysis and minerals. However these differences did not appear to follow any pattern. Percentage moisture in this study ranged between 90.57-93.28% and the USDA database states that *Capsicum spp.* percentage moisture ranges from 87.74-93.89% The average dietary fiber in the four varieties was higher (4.51-7.09%) in comparison to previously studied *Capsicum* spp. in the USDA database, which ranged from 1.5-2.8%. The percentage protein of whole fruit for the four studied varieties ranged from 0.53-0.86% and was similar to that of the *Capsicum* spp. in the USDA nutrient database with protein ranging from 0.86-2.00%. The most prevalent minerals were potassium (99.23-122.93 mg/100g), phosphorous (13.39-20.03 mg/100g), and calcium (1.61-2.85 mg/100g). When compared to the USDA nutrient database for *Capsicum* spp., the calcium (Ca) and potassium (K) content were lower in the four Puerto Rican varieties. The mineral content in our four improved varieties is not a significant source in the daily human diet.

Table 11: Means of proximal analysis and mineral content of four Puerto Rican varieties of *Capsicum chinense*.

	Amanecer	Pasión	Carnaval	Bonanza	F -LSD
Proximal analysis					
Moisture (%)	92.08^{b}	90.57^{a}	93.28^{c}	92.34^{b}	0.63
Protein (%)	0.53^{a}	0.86^{d}	0.65^{c}	0.63^{b}	0.02
Fat (%)	0.06^{a}	0.12^{b}	0.05^{a}	0.12^{b}	0.02
Dietary Fiber (%)	4.51^{a}	7.09^{b}	4.68^{a}	5.16^{a}	0.66
Ashes (%)	0.57^{b}	0.70^{c}	0.48^{a}	0.54^{b}	0.03
Carbohydrates (%)*	5.66	7.48	5.71	6.59	-
Energy (kcal/100g)**	25.3	34.44	25.89	29.96	-
Minerals (mg/100g)					
Al	0.11^{ab}	0.16^{b}	0.07^{a}	0.08^{ab}	0.07
B	0.06^{a}	0.1^{b}	0.05^{a}	0.06^{a}	0.01
Ca	2.75^{b}	2.85^{b}	1.61^{a}	2.76^{b}	0.55
Cu	0.02^{a}	0.02^{b}	0.01^{a}	0.02^{a}	0.005
Fe	0.37^{a}	0.41^{a}	0.2^{a}	0.25^{a}	0.22
K	99.23^{a}	122.93^{b}	116.52^{b}	114.46^{ab}	1.66E+01
Mg	0.0018^{b}	0.0012^{ab}	0.00081^{a}	0.00097^{ab}	0.0008
Mn	0.02^{a}	0.05^{c}	0.03^{ab}	0.04^{bc}	0.016
Mo	0.00012^{a}	ND	ND	0.00075^{b}	0.0004
Na	0.96^{a}	1.33^{a}	1.04^{a}	1.62^{a}	1.57
P	14.49^{a}	20.03^{b}	13.39^{a}	13.71^{a}	1.14
Zn	0.03^{a}	0.08^{b}	0.03^{a}	0.01^{a}	0.02

Means in the same row with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test.
F-LSD= Fishers least significant difference at the 0.05 probability level.
*The carbohydrates were calculated by difference from the proximal analysis.
**Calories calculated with the 4-4-9 method using the protein, carbohydrates and fat content.

The percentage of ash ranged between 0.39 to 0.59% of fresh weight (FW) in the whole fruit and 0.45 to 0.71% FW in the pericarp (Table 12). The placenta and seeds contained a lower percentage ash then the pericarp itself. Amanecer, Pasión, Carnaval and

Bonanza had a 13.5, 13.06, 15.18 and 16.4% increase, respectively, in percentage ash by removing the placenta and seeds.

Percentage protein ranged between 0.85 to 1.25% in the whole fruit and 0.77 to 1.21% in the pericarp. There was no significant difference in percentage ash between pericarp and whole fruit within a variety. On both a whole fruit and pericarp basis, Pasión had the highest percentage protein and Amanecer usually had the lowest percentage. This means that the placenta and seeds do not contribute a significant amount of protein.

The percentage fat ranged between 0.16 to 0.37% in the whole fruit and 0.07 to 0.28% in the pericarp. The percentage fat tended to be higher in whole fruit compared to the pericarp, especially in Amanecer and Pasión. Bonanza had one of the highest percentages of fat. The fat content in the pericarp versus the whole fruit was significantly different in the case of Pasión, while this difference was not observed in whole fruits versus pericarps of Carnaval and Bonanza. However, there were significant differences between each of these later varieties for both whole fruit and pericarp. If we compare this to the placenta with seeds and pericarp ratio, Pasión and Amanecer had the highest placenta content, 11.95 and 11.75 % by weight, respectively, while Carnaval and Bonanza had a 7.38 and 8.4% respectively.

Seeds had a much greater percentage of fat (3.4-4.4%) on a fresh weight basis than did the pericarp (Table 12, 13). These results are comparable to a previous study in Peruvian accessions where the whole fruit fat content depended on the seed to pericarp ratio (Meckelmann et al., 2013). The author also asserts that high fat content is correlated with high content of vitamin E.

Table 12: Percentage of ash, protein and fat in fresh whole fruit and pericarp of four varieties of *Capsicum chinense.*

Variety		Ash[1]	Protein[1]	Fat[1]
Amanecer	Whole fruit	0.39[a]	0.85[ab]	0.18[b]
Pasión	Whole fruit	0.51[bc]	1.25[e]	0.33[c]
Carnaval	Whole fruit	0.55[bc]	0.90[bc]	0.16[b]
Bonanza	Whole fruit	0.59[bcd]	0.94[cd]	0.37[c]
Amanecer	Pericarp[2]	0.45[ab]	0.77[a]	0.07[a]
Pasión	Pericarp[2]	0.59[bcd]	1.21[e]	0.13[ab]
Carnaval	Pericarp[2]	0.64[cd]	0.79[a]	0.09[ab]
Bonanza	Pericarp[2]	0.71[d]	1.00[d]	0.28[c]
F-LSD		0.13	0.07	0.09

Means in the same column with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test.
F-LSD = Fisher's least significant difference at the 0.05 probability level.
[1]Percentage by weight on a fresh basis
[2]Seeds and placental tissue removed

Table 13: Means of percentage of moisture and fat (fresh and dry weight basis) in seeds of three varieties of *Capsicum chinense*.

		Fat (%)	
	Moisture Content (%)	Dry weight basis	Fresh weight basis
Amanecer	55.2[a]	9.9[c]	4.4[b]
Pasión	55.9[b]	8.2[b]	3.6[a]
Bonanza	55.8[ab]	7.8[a]	3.4[a]
F-LSD	0.63	0.22	0.15

Means in the same column with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test
F-LSD=Fisher's least significant difference at the 0.05 probability level

5. 6. Antioxidant properties

5. 6. 1. Ascorbic acid (AA)

Compared to a previous study done on various *C. chinense* ascensions from different countries of origin, including Puerto Rico where AA content varied from 30-70 mg/100g FW (Antonious et al., 2009), the tested varieties had higher AA contents (68.1-115.8 mg/100g FW) (Table 14). The results are comparable to a study by Howard (2000) with mature habanero pepper (*C. chinense*) where AA content ranged from 115.16-122.02 mg/100g. Campos and contributors (2013) studied various genotypes of Habanero pepper and observed content that ranged between 187.24 and 281.73 mg/100g of sample. Peppers are considered a good source of AA and as the fruit matures the AA content increases (Martinez et al., 2005). The Recommended Dietary Allowance (RDA) of AA for adult men is of 90 mg per day and 75 mg for adult women (Institute of Medicine, 2001). The results in this study indicate that 100 g of Amanecer or Carnaval can provide the RDA for adults.

The AA content increased significantly in all varieties as the fruit turned from green (23.78-47.57 mg/100g) to red (92.49-148.86 mg/100g) (Table 15). There was also a significant positive correlation (r=0.85) between AA content and percentage reducing sugar. As the fruit changed from green to red the percentage reducing sugar and AA content increased. AA content, glucose metabolism and light exposure are related. As the fruit matures both AA and reducing sugars increase (Fox, Del Pozo-Insfran, Joon, Sargent, & Talcott, 2005; Mozafar, 1994).

Table 14: Ascorbic acid (AA) content (mg/100g of fresh weight [FW]) of four varieties of *Capsicum chinense* (green and red mixed).

Variety	AA mg/100g FW
Amanecer	91.4[ab]
Pasión	68.1[a]
Carnaval	115.8[b]
Bonanza	73.3[ab]
F-LSD	40.6

Means with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test.
F-LSD=Fisher's least significant difference at the 0.05 probability level.

Table 15: Ascorbic acid (AA) and percentage reducing sugars (RS %) content on a fresh weight basis in green and red fruit of four sweet chili pepper varieties.

Variety	Fruit color stage	AA mg/100g FW	RS % FW
Amanecer	Green	47.57[c]	2.23[d]
Pasión	Green	23.78[a]	1.32[a]
Carnaval	Green	32.48[b]	1.74[b]
Bonanza	Green	31.71[ab]	2.15[c]
Amanecer	Red	148.86[e]	3.39[f]
Pasión	Red	99.98[d]	3.24[e]
Carnaval	Red	95.03[d]	2.26[d]
Bonanza	Red	92.49[d]	3.74[g]
F-LSD		2.71	0.07

Means with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test
F-LSD= Fisher's least significant difference at the 0.05 probability level

5. 6. 2. β -carotene:

In each of the four improved Puerto Rican varieties in this study, the β -carotene content greatly increased as fruit color changed from green to red fruit (Table 16). This same pattern was seen in *C. annuum* (Navarro, Garrido, Flores, & Martinez, 2010), and other *C. chinense* varieties (Menichini et al., 2009). At the green color stage there were no differences in β -carotene content among the four varieties. Once the fruits obtained a red color there were very large differences in β -carotene among all four varieties.

Using the Antonious et al. (2009) method, the average β -carotene content in our four improved varieties was 0.02 mg/g for green fruit and 0.31 mg/g for red fruit. This was significantly lower than the Puerto Rican *C. chinense* varieties in Antonious et al. (2009) in which the overall β -carotene content of mature fruit ranged between 2-5 mg/g of fresh fruit.

Because of the low β -carotene contents obtained with the Antonious et al. (2009) method, a second method, a simple pigment extraction with measurement of the optical density (Nagata & Yamashita, 1992), was used. The β-carotene content in red fruit of Pasión (0.58 and 0.57 mg/g) and Bonanza (0.17 and 0.19 mg/g) were similar using both methods for red fruit (Table 16). Difference between the study presented here and that of Antonious et al (2009) could be due to genetic differences between the genotypes of the two studies or possibly due to the stage of maturity.

Hornero-Mendez and contributors (2000), studied the changes in carotenoid biosynthesis in five pepper cultivars *(C. annuum)* during ripening. The results show a characteristic pattern for the *Capsicum* genus. During ripening, chloroplast pigments (lutein and neoxanthin) decreased in concentration and disappeared. Meanwhile β -carotene increased in concentration and other pigments were biosynthesized de novo (zeaxanthin and

β -cryptoxanthin, capsanthin, capsorubin, casanthin-5,6-epoxide and cucurbitaxanthin). Zeaxanthin stands out from the rest of the pigments, revealing the importance of this pigment as a branching point in the carotenoid biosynthesis in *Capsicum* (Guil-Guerrero, Martínez-Guirado, del Mar Rebolloso-Fuentes, & Carrique-Pérez, 2006; Hornero-Méndez, Gómez-Ladrón De Guevara, & Mínguez-Mosquera, 2000) During fruit ripening the fruit changes from green to orange to red and this is attributed to a decrease in chlorophyll (green color), and synthesis of β-carotene (yellow-orange color) and xanthophyl (red color) (Cervantes-Paz et al., 2014; Guil-Guerrero et al., 2006; Hornero-Méndez et al., 2000; Howard, Talcott, Brenes, & Villalon, 2000; Reeves, 1987). The β-carotene concentrations in *Capsicum* species (*chinense, annuum, frutescens*) increased during maturity (Howard et al., 2000). In *C. chinense* varieties, β-carotene increased from 62.7 mg/100 g to 362 mg/100 (Menichini et al., 2009).

Table 16: β -carotene content in green and red fresh fruit of four varieties of *Capsicum chinense*.

	Fruit color stage	(mg/g) FW Antonious	Comparison of methods (mg/g)FW Antonious	Nagata
Amanacer	Green	0.01[a]	-	-
Pasión	Green	0.07[b]	0.06	0.02
Canaval	Green	0.01[a]	-	-
Bonanza	Green	0.01[a]	0.06	0.01
Amanacer	Red	0.23[d]	-	-
Pasión	Red	0.57[f]	0.57	0.58
Canaval	Red	0.14[c]	-	-
Bonanza	Red	0.32[e]	0.19	0.17
F-LSD		0.008	-	-

Means with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test
F-LSD=Fisher's least significant difference at the 0.05 probability level

5. 6. 3. Phenols, flavonoid and antioxidant activity and capacity

5. 6. 3. 1. Phenolic compounds:

The varieties contained an overall mean phenolic content of 103.56 and 106.8 GAE mg/100g FW (fresh weight basis) in whole fruits and pericarps respectively (Table 17). Amanecer had the greatest phenol content while Bonanza and Carnaval generally had the lowest phenolic content. The four varieties had significantly higher phenolic content (721.07-1074.1 mg GAE /100g DW) than other varieties of *C. chinense* studied by Campos and contributors (2013) in which the total phenol content ranged from 20.54 to 20.75 mg (GAE) /100g DW of sample, with a higher content in red fruit in comparison with orange and yellow fruit.

As fruit turned from green to red, phenol content increased in all varieties (Table 18). The average increase in phenol content between green and red fruit was 32.7 mg GAE/g FW, and increase of about 9%. In a study done by Siddiqui and contributors (2012) the changes of antioxidant content in Habanero pepper at various maturity stages resulted in an initial increase of phenolic content from 140 to 330.79 mg cathecol equivalents (CE)/100g FW at 7 to 42 days after fruit set. Afterwards there was a decrease to 273.36 mg CE/100g FW at 63 days after fruit set. The increase in phenolic content in the four improved varieties was comparable to the maximum content recorded (42nd day) in Habanero pepper by Siddiqui et al (2010).

Table 17: Mean phenolic content, flavonoid content; antioxidant activity and antioxidant capacity of freeze dried samples of whole fruit and pericarp of four varieties of sweet chili pepper.

		Phenols		Flavonoids			Antioxidant activity (DPPH method)		Antioxidant capacity (ORAC method)	
		(mg GAE /100g)		(mg QE /100g	(mg QE /100g	(mg RE /100g	(μ M TEAC /g	(μ M TEAC /g	(μ mol TE/g	(μ mol TE/g
Variety		FW	DW	FW)	DW)	FW)	FW)	DW)	FW)	DW)
Amanecer	Whole fruit	137.06^{f}	1074.1^{d}	21.14^{b}	151.97^{a}	88.03^{a}	57.80^{bc}	455.83^{ab}	116.33^{b}	1115.77^{b}
Pasión	Whole fruit	110.26^{d}	982.7^{c}	58.30^{c}	385.05^{b}	187.41^{b}	42.05^{ab}	312.53^{a}	246.61^{c}	2197.98^{d}
Carnaval	Whole fruit	89.28^{c}	940.18^{c}	22.27^{b}	165.65^{a}	46.40^{a}	45.27^{ab}	461.3a^{b}	131.21^{b}	1496.09^{c}
Bonanza	Whole fruit	77.66^{b}	721.07^{ab}	15.49^{a}	119.62^{a}	91.29^{a}	55.20^{abc}	312.87^{a}	107.71^{b}	1000.07^{ab}
Amanecer	Pericarp[1]	169.44^{g}	1327.88^{f}	14.56^{a}	114.14^{a}	80.17^{a}	81.75^{c}	913.42^{c}	63.08^{a}	888.10^{a}
Pasión	Pericarp[1]	124.01^{e}	1199.2^{e}	57.28^{c}	465.78^{b}	197.67^{b}	67.62b^{c}	667.89^{bc}	244.56^{c}	2179.72^{d}
Carnaval	Pericarp[1]	69.53^{ab}	792.77^{b}	19.47^{ab}	179.99^{a}	35.47^{a}	76.58^{c}	873.22^{c}	123.44^{b}	1407.55^{c}
Bonanza	Pericarp[1]	64.22^{a}	649.96^{a}	16.01^{ab}	128.28^{a}	81.66^{a}	33.70^{a}	558.70^{abc}	104.95^{b}	974.51^{ab}
F-LSD		9.73	88.84	6.52	87.09	77.5	30.43	270.21	27.43	197.12

Means in the same column with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test
F-LSD=Fisher's least significant difference at the 0.05 probability level
FW= fresh weight basis
DW= dry weight basis
GAE= gallic acid equivalents
QE= quercetin equivalents
RE= rutin equivalents
TEAC=Trolox equivalents antioxidant capacity
TE= Trolox equivalents
[1] seeds and placental tissue removed

Table 18: Mean phenolic content, flavonoid content, and antioxidant activity on a fresh weight basis in whole green and red fruit samples of four varieties of *Capsicum chinense*.

Variety	Fruit color stage	Phenols[1] (mg GAE /100g)	Flavonoid [2] (mg QE /100g)	Flavonoid[3] (mg RE /100g)	Antioxidant activity[4] (μ M TEAC /g)
Amanecer	Green	344.75[a]	29.34[c]	23.41[c]	60.14[c]
Pasión	Green	404.88[d]	71.35[d]	61.6[d]	104.03[g]
Carnaval	Green	342.64[a]	22.39[a]	17.09[a]	98.23[f]
Bonanza	Green	336.21[a]	24.32[b]	18.85[b]	74.04[d]
Amanecer	Red	385.79[c]	79.49[e]	69.00[e]	79.06[e]
Pasión	Red	445.73[e]	328.16[g]	295.06[g]	28.78[a]
Carnaval	Red	361.37[b]	80.37[e]	69.79[e]	97.00[f]
Bonanza	Red	366.49[b]	152.08[f]	134.98[f]	46.94[b]
F-LSD		16.01	1.69	1.54	2.89

Means in the same column with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test

F-LSD=Fisher's least significant difference at the 0.05 probability level

[1] GAE= gallic acid equivalents

[2] QE= quercetin equivalents

[3] RE= rutin equivalents

[4] TEAC=Trolox equivalents antioxidant capacity

Table 19: Total phenolic content of fruit components (seed, placenta and pericarp) in green and red fruits of Amanecer.

Fruit component	Fruit color stage	Phenolics (mg GAE/100g FW)
Seed	Green	125.6[a]
Placenta	Green	258.87[d]
Pericarp	Green	175.2[c]
Whole Fruit	Green	155.29[b]
Seed	Red	273.16[de]
Placenta	Red	285.6[e]
Pericarp	Red	257.27[d]
Whole Fruit	Red	256.86[d]
F-LSD		16.93

Means with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test.

F-LSD= Fisher's least significant difference at the 0.05 probability level

FW=fresh weight of fruit component

In order to better determine where phenols are located in sweet chili pepper fruit, green and red fruits of Amanecer were separated into their components (seed, placenta and pericarp) before homogenization. There was a significant increase in phenolic content in each fruit component as the fruit color changed from green to red (Table 19). In both green and red fruit the placenta had a higher content of phenols compared to the pericarp. This characteristic is similar to a previous study in Habanero pepper in which the total phenolic content was mostly found in the placenta (27 g GAE/100g FW) of red fruit resulting in a 150% increase in comparison with the pericarp (~6 g GAE/100g FW) (Castro-Concha et al., 2014).

In red Amanecer fruit, 84% of the total phenolic content in fruit is attributed to the pericarp and 7% is attributed to the placenta, but the content in the placenta per gram of tissue is significantly higher than the content in the pericarp per gram of tissue (Table 20). The variation between phenolic content in the various fruit components will be related to the relative percentage of total weight that the placenta and pericarp contribute in each individual variety (Table 20).

Table 20: Percentage of whole fruit weight (fresh weight) contributed by the seed, placenta and pericarp in four varieties of *Capsicum chinense*.

	Percent (%)		
Variety	Seed	Placenta	Pericarp
Amanecer	6.81[b]	5.29[b]	87.9[a]
Bonanza	6.91[b]	4.33[a]	88.8[a]
Pasión	7.20[b]	5.58[b]	87.2[a]
Carnaval	2.83[a]	5.80[b]	91.4[b]
F-LSD	1.41	0.82	1.77

Values in the same column with a common letter are not different at the 0.05 probability lever according to Fisher's Least Significant Different test.
F-LSD=Fisher's least significant difference at the 0.05 probability level

5. 6. 3. 2. Flavonoid content

The flavonoid content both the whole fruit and pericarp of Pasión was significantly higher than that of the other varieties with 465.78 and 385.05 mg (QE) /100g DW in the pericarp and whole fruit, respectively (Table 17). Estimates of flavonoid content were somewhat higher when measure as milligrams of rutin equivalents (RE) compared to milligrams of Quercetin Equivalents (QE). The total flavonoid content in the four varieties ranged from 46.40 to 187.41 mg RE/100g FW (Table 17) which is comparable to Habanero varieties (*C. chinense*) studied by Menichini et al. (2009) and Siddiqui et al. (2012). In general, flavonoid content on a FW or DW basis did not change in whole fruit compared to fruit with seeds and placenta removed (pericarp). However, flavonoid content increased in all varieties as fruit color changed from green to red (Table 18). Pasión had the highest flavonoid content whether considering whole fruit, pericarp, green fruit or red fruit (Tables 17 and 18).

In a study done by Siddiqui and contributors (2013) the changes of antioxidant content in Habanero pepper at various maturity stages resulted in an initial increase of flavonoid content from 45.11 to 137 mg RE/100g. Menichini and contributors (2009) expressed flavonoid content in quercetin equivalents (QE) and found a decrease in QE (138 to 45 mg QE/100g) as fruit matures from green to red.

Vera-Guzmán, Chávez-Servia, & Carrillo-Rodríguez (2011) found that flavonoid content was affected by maturity and pigment content; there was a positive correlation between flavonoid concentration and red color where redder fruit presented higher flavonoid concentration. Flavonoid content for Habanero pepper ranged from 45 to 138 mg QE /100g FW from immature and mature fruit respectively (Menichini et al., 2009).

Overall, our results for flavonoid content (119.62-385.05 mg QE/100g DW of whole fruit) are higher than the 85 accessions of Peruvian *C. chinense* studied by Meckelmann and contributors (2014) in which the highest content was of 26.6 mg QE /100g DW.

5. 6. 3. 3. Antioxidant activity (DPPH method)

Antioxidant activity (DPPH method) on a whole fruit DW basis was significantly lower (with the exception of Bonanza) than in the pericarp, suggesting that the highest antioxidant capacity can be found in the pericarp of the fruit (Table 17). Bonanza had the lowest antioxidant activity (33.70 μM TEAC/g FW) in the pericarp while the other varieties were not different.

To determine the effects of fruit components (seed, placenta and pericarp) and fruit color stage on antioxidant capacity, both red and green fruits of Amanecer fruit were separated into seed, placenta, and pericarp and extracts were prepared. Antioxidant capacity more than doubled in red fruit compared to green fruit (Table 21). In both green and red fruit, seeds contributed the lowest antioxidant activity, followed by the placenta and then the pericarp. For the seed and placenta components, there were no significant differences between green and red fruit, but there was a significant increase in antioxidant capacity in the pericarp as the fruit changed from green to red. The results do not agree with a previous study where the antioxidant capacity of the placenta was greater than that of the pericarp of red fruit (Castro-Concha et al., 2014).

In Amanecer the antioxidant properties are found throughout the whole fruit and the variation between varieties can be related to the relative percentage of the weight of the placenta versus the pericarp (Table 20) and contribution of each individual variety.

Table 21: Antioxidant activity (DPPH method) on a fresh weight basis in fruit components (seed, placenta and pericarp) of green and red fruits of Amanecer.

Fruit component	Fruit color stage	μ M TEAC /g of fruit component
Seed	Green	12.85[a]
Placenta	Green	78.99[c]
Pericarp	Green	93.23[d]
Whole fruit	Green	50.04[b]
Seed	Red	14.37[a]
Placenta	Red	72.21[c]
Pericarp	Red	129.13[f]
Whole fruit	Red	117.62[e]
F-LSD		9.92

Means with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test.
F-LSD=Fisher's least significant difference at the 0.05 probability level.

5. 6. 3. 4. ORAC method of antioxidant capacity

For both whole fruit and pericarp extracts of Pasión, prepared with 80% methanol, presented higher antioxidant capacity in both whole fruit and pericarp (246.61 and 244.56 μ mol TE/g FW respectively) expressed in Trolox equivalents. The varieties that presented the lowest antioxidant capacities were Amanecer and Bonanza, 116.33 and 107.71 μ mol/g FW respectively in the whole fruit with no significant difference between them. There are no previous reports of antioxidant capacity determined by the ORAC method for *Capsicum chinense*. In the USDA database the mean total-ORAC ranged from 615 to 1043 μ mol TE/100g FW in sweet green, red and yellow peppers (*C. annuum*). In *Capsicum annuum* (Jalapeño and Serrano) the ORAC values ranged from 42.4-58.7 mmol TE/100g DW (Alvarez-Parrilla, De La Rosa, Amarowicz, & Shahidi, 2011).

When comparing the overall means of pericarp tissue and whole fruit there is no significant difference in ORAC antioxidant activity between the tissue samples when expressed in DW. After converting to FW there was a significant difference between the overall mean of the tissue samples. In both cases the whole fruit contains higher antioxidant capacity than the pericarp, meaning that the highest antioxidant capacity can be found in the placenta and seeds. The average results in FW and DW are higher than previously studied green and red peppers (Lutz, Hernández, & Henríquez, 2015; Ou et al., 2002).

5. 6. 4. Correlation between phytochemical composition and color measurements

Color measurements were taken on the same fruits evaluated for phytochemical composition of fresh green and red fruit (Table 21). These fruit samples were not the same as those used to measure color in part 5. 1. Thus the color values reported in table 21 are somewhat different from those previously reported in table 7.

Hue angle has a negative and significant correlation with β-carotene meaning that fruit with lower Hue values will have higher β-carotene concentrations. For example Pasión had a low brightness in red fruit (28.96) and had the highest β-carotene content (0.57 mg/g). L* (lightness and darkness) has a significant negative correlation with β-carotenoids (r=-0.84), stating that higher β-carotene content is related to darkness of pericarp. AA, flavonoid and phenols were also strongly negatively correlated with L* (luminosity) (Table 22). The values of these parameters increased as luminosity decreased, meaning that the darkest fruits have the highest phytochemical content.

Chroma was negatively correlated with phenolic compounds, red Pasión fruit had the highest phenolic content and the lowest chroma value, meaning that the least saturated fruit had the highest phenolic content.

Table 22: Color measurements in green and red fruit of four varieties of *Capscium chinense* harvested for phytochemical analysis.

Variety	Fruit color stage	L*	Hue angle	Chroma (C*)
Amanecer	Green	61.73^{d}	101.88^{d}	44.98^{b}
Pasión	Green	51.24^{c}	106.66^{e}	41.98^{ab}
Carnaval	Green	59.11^{d}	102.9^{d}	48.81^{c}
Bonanza	Green	61.49^{d}	100.9^{d}	42.07^{a}
Amanecer	Red	32.05^{a}	31.95^{b}	49.17^{c}
Pasión	Red	34.24^{a}	28.96^{a}	40.3^{a}
Carnaval	Red	42.40^{b}	35.61^{c}	54.54^{d}
Bonanza	Red	35.93^{a}	31.52^{b}	49.77^{c}
F-LSD		4.62	1.99	3.49

Means with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test
F-LSD=Fisher's least significant difference at the 0.05 probability level

Table 23: Pearson correlations (r) coefficients among β -carotene, ascorbic acid, flavonoid content, phenolic content, antioxidant activity and color chromatic coordinates in the fresh green and red *C. chinense* varieties.

	Chromatic coordinates		
Phytochemical compounds	L*	Hue angle	Croma (C*)
β -carotene	-0.84**	-0.80**	-0.13^{NS}
Ascorbic Acid	-0.83**	0.32^{NS}	-0.32*
Phenolic compounds	-0.77**	-0.59**	-0.34*
Flavonoids	-0.68**	-0.69**	-0.05 ^{NS}
Antioxidant activity (DPPH method)	0.40^{NS}	0.47*	0.35 ^{NS}

^{NS} non significant at p>0.05
* significant at p<0.05
** significant at p<0.01

5. 7. Capsaicinoids

The average retention times (RT) were 5.70 and 9.19 for capsaicin and dihydrocapsaicin respectively (Table 24), which agreed with the RT of the standard peaks. Pasión had the highest concentration of capsaicin (on a DW basis) as well as the highest SHU value. Since there were no differences among varieties in concentration of dihydrocapsaicin, the differences in SHU were due to differences in capsaicin. When looking at content of capsaicinoids on a fresh weight basis (Table 24), Pasión again had the highest amount of capsaicin (0.00054 mg/g FW), while Carnaval had the lowest amount. Pasión together with Amanecer tended to have higher amounts of dihydrocapsaicin compared to Carnaval and Bonanza. The concentrations and amounts of capsaicinoids observed in these *C. chinense* varieties were significantly lower than in Puerto Rican varieties studied by Antonious, Berke, & Jarret, (2009). They found that in the highest capsaicin plus dihydrocapsaicin content in Puerto Rican varieties was 0.2 mg/g fruit FW. Our results converted to FW were between 0.0003-0.001 mg/g fresh fruit.

Table 24: Mean capsaicin and dihydrocapsaicin peak retention times, area under the peak, concentration (ppm) on a dry weight basis (DW), content (μ g/g) on a fresh weight basis (FW) and the SHU of four varieties of Puerto Rican *Capsicum chinense*

Variety	Capsaicin				Dihydrocapsaicin				SHU
	Retention time (min)	Area under the peak	Capsaicin content in sample (μ g/g) DW	(μ g/g) FW*	Retention time (min)	Area under the peak	Dihydrocapsaicin content in sample (μ g/g) DW	(μ g/g) FW*	
Amanecer	5.61^{a}	190959^{a}	0.22^{a}	0.28^{b}	9.14^{a}	87554 ^{c}	0.13^{a}	0.22^{b}	45.48 ^{a}
Pasión	5.68^{a}	404665^{b}	0.48^{b}	0.54^{c}	9.10^{a}	74958^{bc}	0.11 ^{a}	0.17^{ab}	80.22 ^{b}
Carnaval	5.85^{a}	143223^{a}	0.16^{a}	0.14^{a}	9.28^{a}	41640^{a}	0.07^{a}	0.14^{a}	34.55 ^{a}
Bonanza	5.66^{a}	230302^{a}	0.27^{a}	0.29^{b}	9.2^{a}	61955^{ab}	0.10 ^{a}	0.15^{a}	48.66 ^{a}
F-LSD	0.52	94673	0.11	0.10	0.21	22629	0.06	0.06	20.7

Means in the same column with a common letter are not different at the 0.05 probability level according to Fisher's Least Significant Different test
F-LSD=Fisher's least significant difference at the 0.05 probability level
SHU = Scoville heat units
*FW value is calculated using the moisture content of the freeze-dried samples

Because the results of this study were so different from other Puerto Rican varieties previously studied, the efficiency of the extraction procedure (Collins et al., 1995) was validated. A jalapeno sample (*C. annuum*) was processed and extracted in the same way as the *C. chinense* samples. The jalapeño sample was spiked using a standard of capsaicin and dihydrocapsaicin. Jalapeño is known to have a SHU of approximately 3500 to 10,000 (Boning, 2010; Marshall et al., 1981). Table 25 gives the concentration of capsaicin and dihydrocapsaicn following the Collins et al., (1995) method. These values give a SHU of 5672, which is in the range of the known SHU values for jalapeño peppers. Jalapeño was spiked with a known concentration of 2.5 ppm of capsaicin and dihydrocapsaicin in acetonitrile and the recovery percent was very high: 116.65 and 98.32% respectively, thus validating the protocol (Table 25).

The Capsaicinoid content varies on the maturity of the fruit and color of fruit (Pino et al., 2007). Menichini and contributors (2008) determined the capsaicin and dihydrocapsaicin content for *C. chinense* (Habanero) and found 4363 and 2498 μ g/g FW respectively in mature fruit, and 1071 and 2498 μ g/g FW (1.07 and 2.5 mg/g) in immature fruit. Total capsaicinoids ranged from 41.8 to 65.9 mg/g DW for *C. chinense* originating from Yucatan (Pino et al., 2007). In a study comparing ascensions from various countries the highest capsaicinoid content was 1.36 mg/g FW and the lowest content came from the Puerto Rican accessions with 0.2 mg/g FW (Antonious, et al., 2009). Our four varieties contained lower concentrations of capsaicinoid and dihydrocapsaicinoids (Table 24) compared to Habanero pepper, and other Puerto Rican *C. chinense* previously studied (Antonious, et al., 2009; Menichini et al., 2009; Pino et

al., 2007). In sensory tests (data not shown), panelists were unable to detect pungency in samples of the four varieties tested.

Table 25: Percentage recovery of a spiked sample of a reference sample of Jalapeño pepper.

Concentration	Capsaicin (ppm)	Dihydrocapsaicin (ppm)
Jalapeño pepper	32.31	12.38
Spiked added	1.5	1.5
Spiked sample	34.06	13.85
Recovery (%)	116.65	98.32

Values represent the mean of the triplicate. ppm=mg/L

6. CONCLUSION

The four Puerto Rican *C. chinense* varieties have nutritional and physicochemical properties and quality parameters that make these varieties ideal for consumption and commercialization. Carnaval had the heaviest fruit, while Amanecer had the longest fruit, these fruits can be of interest to farmers that want to profit from pepper production. Firmness was not affected as fruit changed from green to red fruit. Color parameters determine that as fruit changes from green to red the luminosity decreases and fruit becomes darker in color. Puerto Rican *C. chinense* is an excellent source of vitamin C. A 100 g sample of sweet chili pepper fruit provides about 120% of the recommended daily intake of vitamin C. The sweet chili peppers in this study contained 361-445 mg/100g of phenolic compounds, which gives it antioxidant potential. The variety Pasión exhibited the strongest antioxidant properties. There is a positive correlation between red color in fruit and bioactive components. This study suggests that the red color of sweet chili pepper provides higher phytochemical content; therefore red fruit, rather than green, might be preferable from the nutritional standpoint. All four tested varieties had very low degrees of pungency (determined by HPLC and sensory analysis) and therefore are appropriate for the Puerto Rican palate, which prefers a flavorful, but not pungent fruit. A more specific experiment should be carried out to compare phytochemical content in placenta and pericarp in the four Puerto Rican varieties to determine the possible contributions each tissue can have on their respective varieties.

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8. APPENDIX

8. 1. Calculations and standard curves

8. 1. 1. Phenolic content

Phenolic content was determined using a standard curve of absorbance at 765 nm vs gallic acid concentration in mg/L (Figure 5). A 800 mg/L stock standard solution was prepared and diluted to obtain various calibration points.

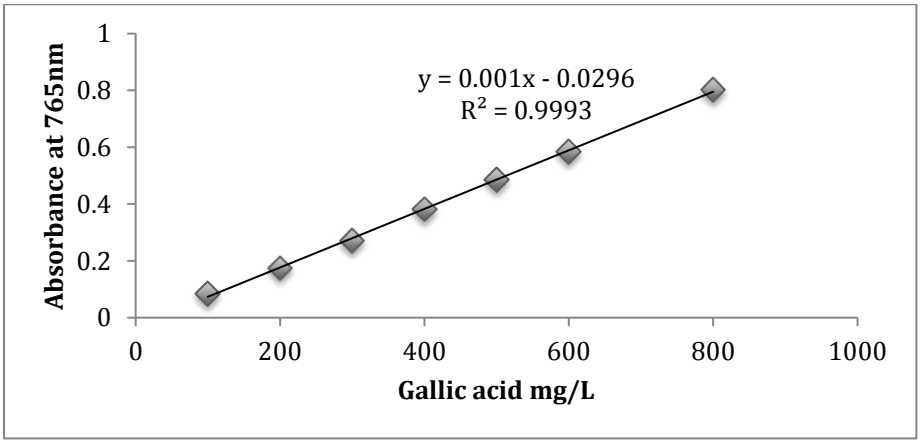


Figure 5: Phenolic content standard curve based on gallic acid equivalents (GAE).

Calculations:

Sample Absorbance: 0.236

Gallic acid mg/L = (0.236+0.0296)/0.001 = 265.6 mg/L

265.6 mg/L (0.005L solvent/0.125g sample) = 10.62 GAE mg/g dry weight

8. 1. 2. Flavonoid content

The Flavonoid content was determined using a standard curve of absorbance vs quercetin equivalents (QE) μ g/ml (Figure 6). A 150 mg/ml stock solution was prepared and diluted to various calibration points.

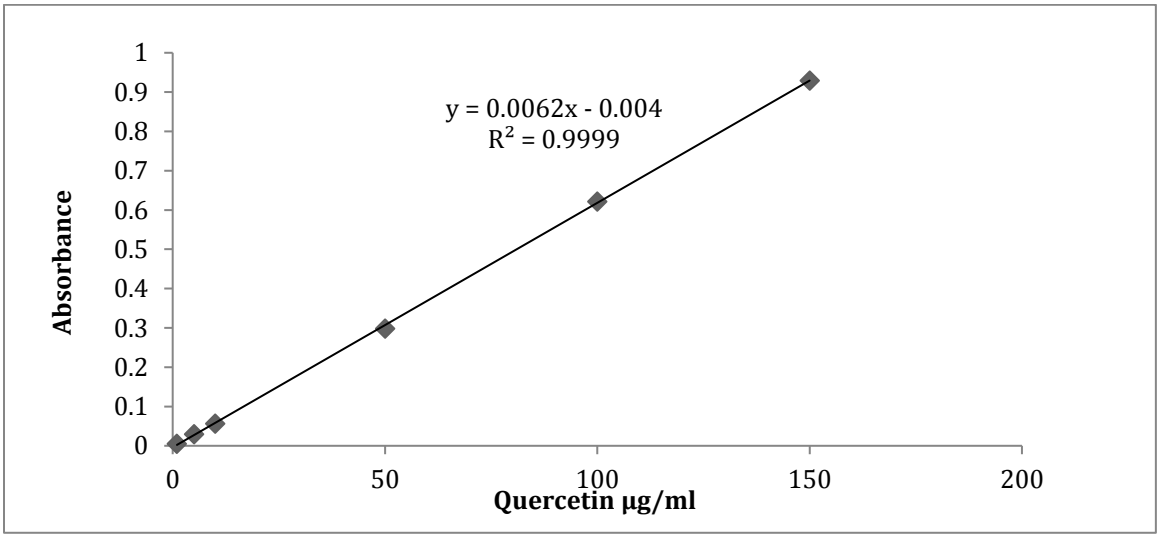


Figure 6: Flavonoid standard curve in quercetin equivalents(QE)

Calculations:

Sample Absorbance: 0.259

Quercetin μ g/ml = (0.259+0.004)/0.0062= 42.42 μ g/ml

42.42 μ g/ml (5mL solvent/0.125g sample) = 1696.77 QE μ g/g dry weight

8. 1. 3. Antioxidant activity

Antioxidant activity was determined using the DPPH method using a standard curve of radical scavenging activity (RSA%) vs Trolox concentration (μ M). The standard Trolox solution (1800 μ M) was diluted to obtain various calibration points. The RSA% was calculated using Equation 9.

Equation 7:

$$RSA\% = 100 * (\frac{Abs_0 - Abs_1}{Abs_0})$$

In which Abs= Absorbance

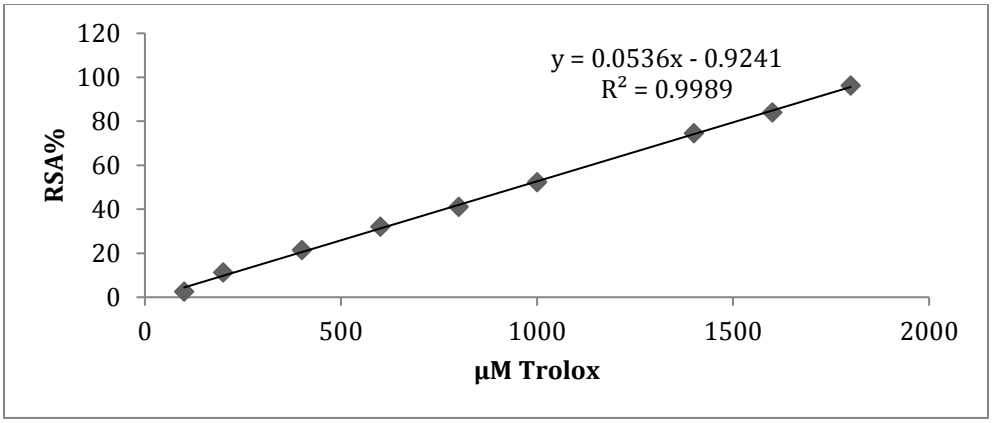


Figure 7: Radical scavenging activity standard curve.

Calculations:

Abs_0 = 0.998, Abs_1 = 0.775, dilution factor (FD) = 40, Solvent vol. = 0.005L, Sample = 0.125 g

$$RSA\ \% = 100 * (\frac{0.998 - 0.775}{0.998}) = 22.34\%$$

μ M Trolox= (22.34+0.9241)/0.536= 434.12 μ M Trolox

434.12 μ mol/L* FD=17364.77 μ mol/L

17364.77 μmol/L*(0.005L solvent/0.125g sample) = 694.6 μmol TE/g dry weight

8. 1. 4. Antioxidant capacity assay

The antioxidant capacity was determined by the $ORAC_{FL}$ method using a standard curve of Trolox Concentration (μ M) vs Standard Net AUC (Figure 8).

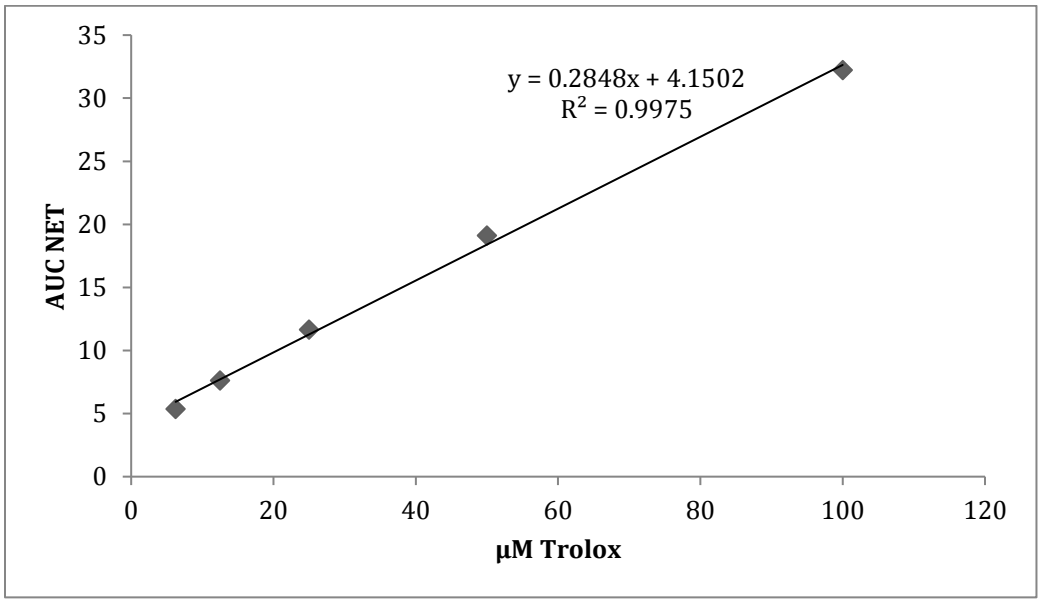


Figure 8: Trolox calibration curve for $ORAC_{FL}$ assay

The Raw data exported by the Ascent™ Software Version 2.6 (Thermo Scientific) in Excel format was expressed in relative fluorescent units (RFU). The 60 raw RFUs for the quadruplicate samples were compared statistically and the outliers were determined by calculating the Z-score of each minute reading. Any data that had a z-score of less than -3 or greater than 3 were considered outliers (data that was more than 3 standard deviations from the mean) and were eliminated. The antioxidant curves were normalized to the blank curve using Equation 10 (Dávalos, Gómez-Cordovés, & Bartolomé, 2004).

The area under the curve (AUC) was calculated using Equation 11 and the Net AUC was determined by subtracting the AUC_{sample} from the AUC_{blank}.

Equation 10:

$$Normalized\ data = \frac{Raw\ Data \times RFU_{blank,t=0}}{RFU_{sample,t=0}}$$

Equation 11:

$$AUC = \left(0.5 + \frac{F1}{F0} + \frac{F2}{F0} + \frac{F3}{F0} + \ldots + \frac{F59}{F0} + \frac{Fi}{F0}\right) * CT$$

In which:

RFU= Relative fluorescent units

F0 = Normalized initial RFU

Fi = Normalized RFU reading at time i

CT = Cycle time in minutes = 1

Final ORAC values were calculated using the regression equation from the corresponding standard curve (y = a + b x) between AUC (Y axis) and Trolox concentration corresponding to the FL decay curve (X axis). The results were expressed in mmol/100g sample using the following formulas:

Dilution factor (FD) = 500, Solvent Volume = 0.005L, Sample = 0.125 g

$$\frac{(AUCNet - Intercept) \times FD \times L_s}{slope * G} = \frac{\mu mol\ TE}{g} DW$$

$$\frac{\mu mol\ TE}{g} DW \times \frac{1mmol}{1000\mu mol} = \frac{mmolTE}{g} DW$$

In which:

L_s = Extract solvent volume

G = grams of freeze dried sample in extract

TE = Trolox Equivalent

8. 1. 5. Total capsaicinoids

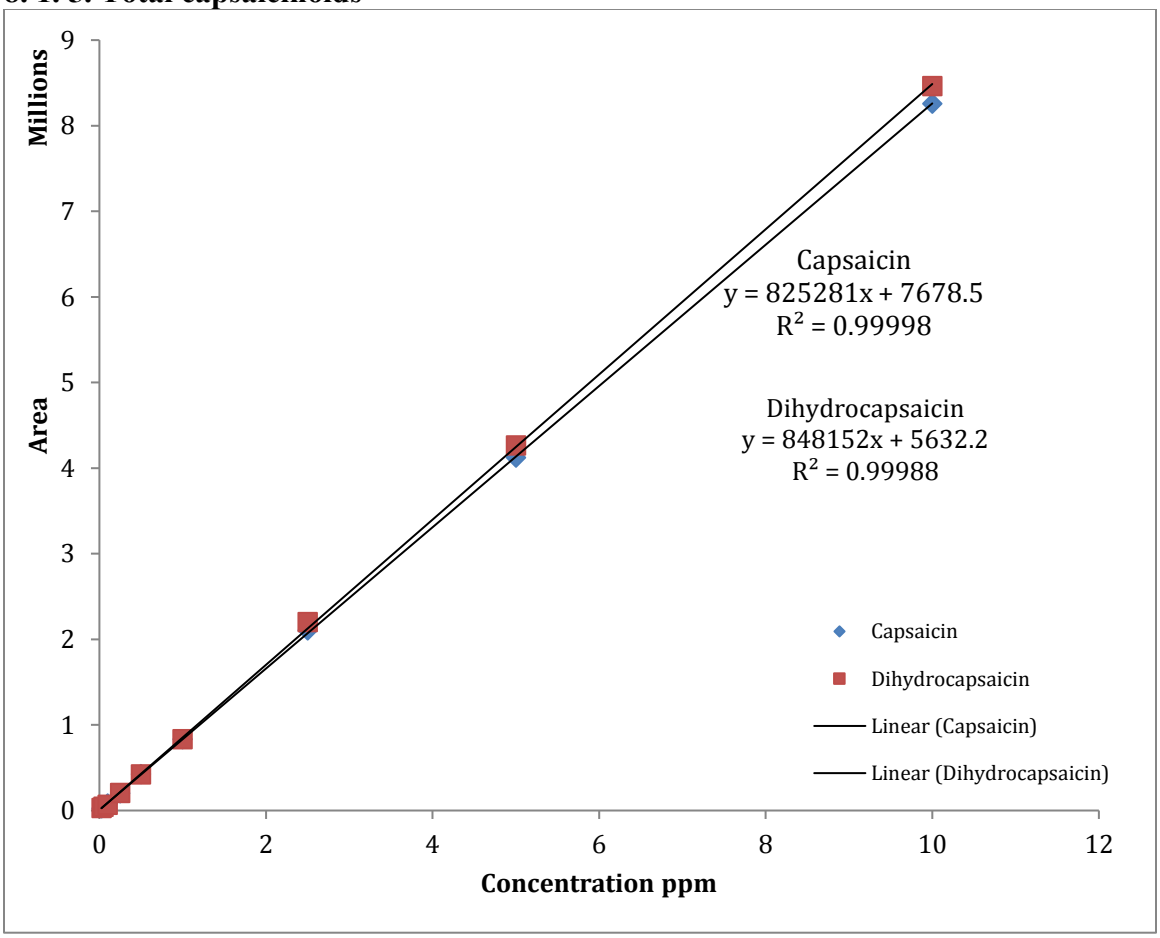


Figure 9: Calibration curve for Capsaicin and Dihydrocapsaicin

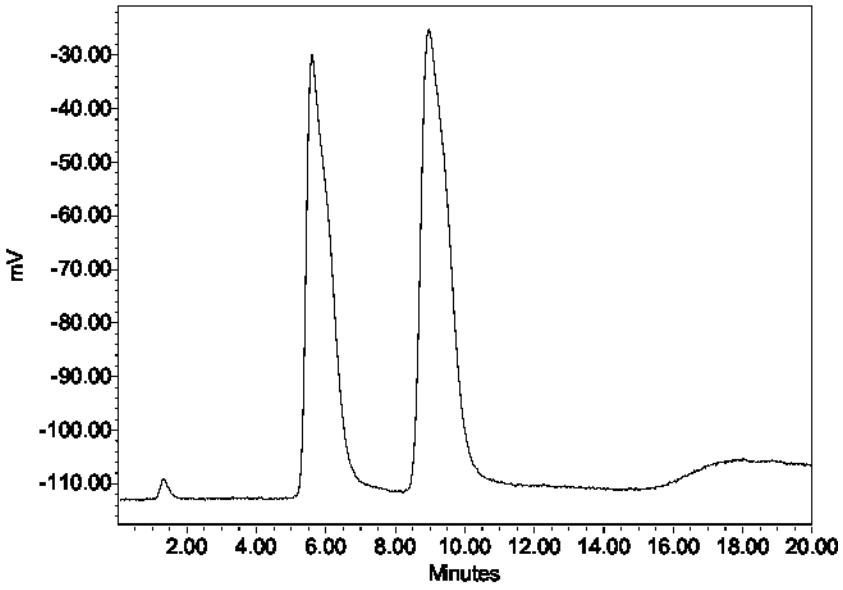


Figure 10: Reference Standard Peaks of Capsaicin and Dihydrocapsaicin for identification

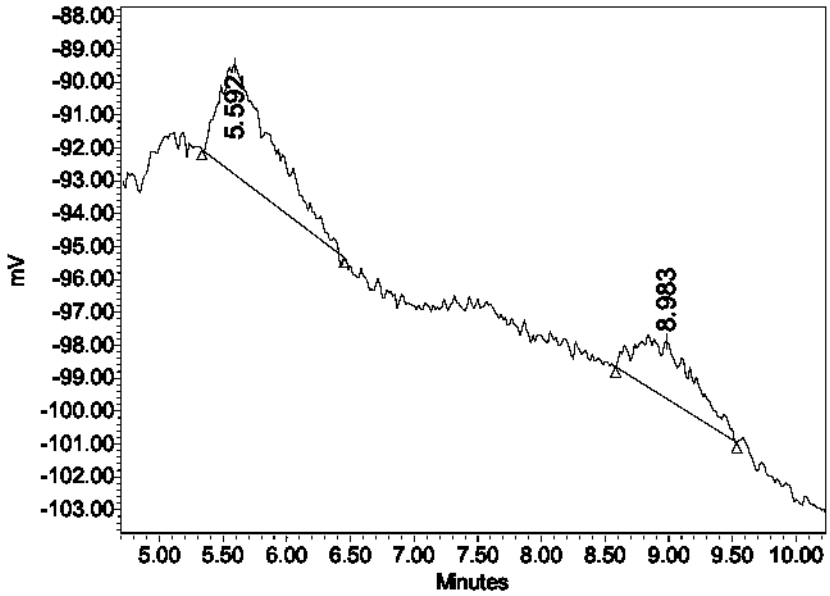


Figure 11: Chromatography peaks and retention times for Capsaicin and Dihydrocapsaicin in Amanecer extract

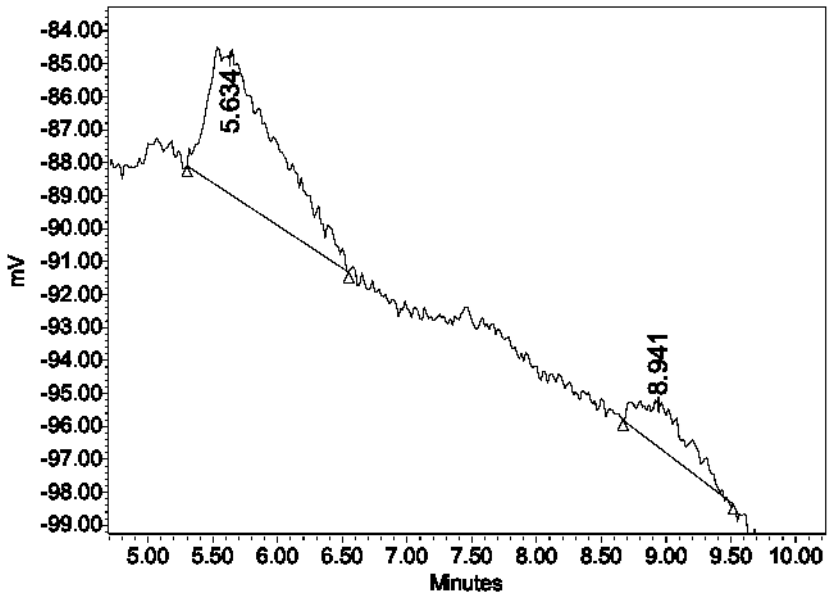


Figure 12: Chromatography peaks and retention times for Capsaicin and Dihydrocapsaicin in Pasión extract

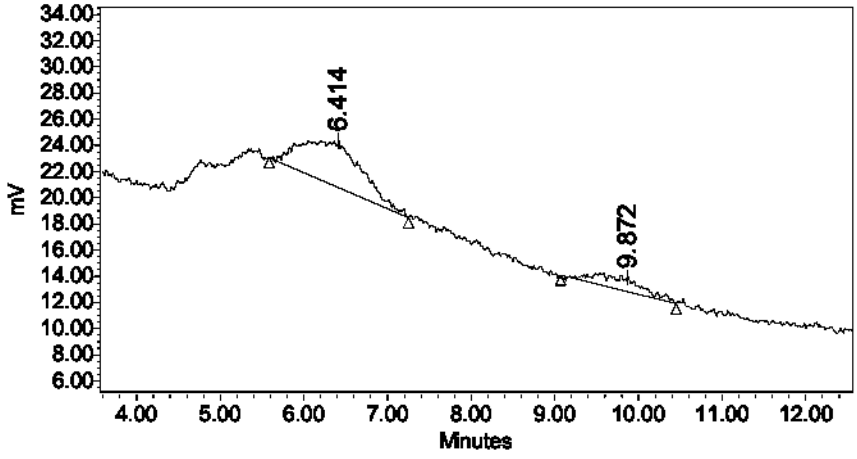


Figure 13: Chromatography peaks and retention times for Capsaicin and Dihydrocapsaicin in Carnaval extract

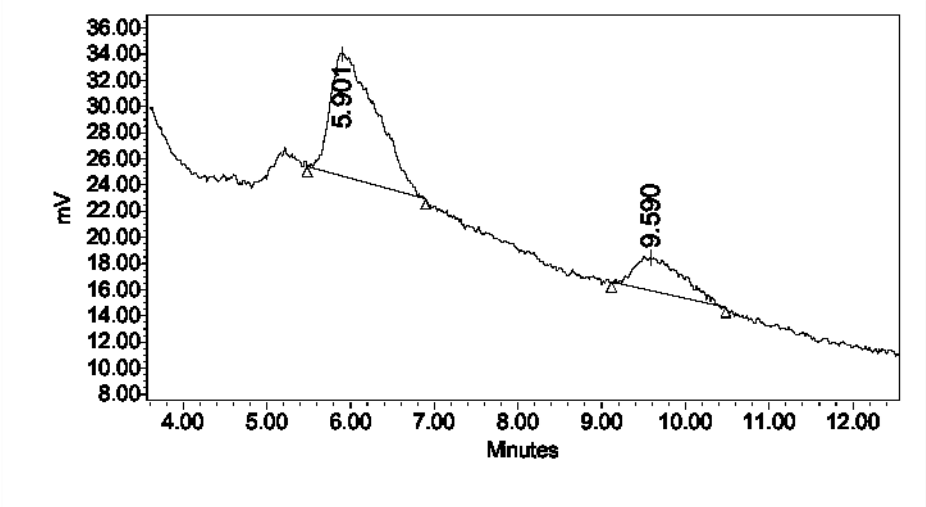


Figure 14: Chromatography peaks and retention times for Capsaicin and Dihydrocapsaicin in Bonanza extract

Table 26: Capsaicinoid standard curve areas

	Capsaicin	Dihydrocapsaicin
ppm*	Area	Area
0.025	24184	28333
0.05	46506	43739
0.1	81361	67133
0.25	202611	205440
0.5	426202	40.5584
1	834367	833093
2.5	2103755	2200164
5	4123867	4263295
10	8257343	8463185

*ppm=mg/L

8. 2. Dry weight to fresh weight conversion

The antioxidant analysis that were done using extracts prepared with freeze dried samples the results were calculated in dry weight (DW) and were converted to fresh weight (FW) (Equation 12) to compare to previous studies in Capsicum spp.

Equation 12:

$$FW = DW * (100 - \%\, Moisture\ Content)/100$$

Table 27: Moisture content % means of the freeze-dried samples

	Moisture Content %
Amanecer	87.24
Pasión	88.78
Carnaval	91.55
Bonanza	89.23

8. 3. Sensory Analysis

Table 28: Calibration and standardization results of second session

	Random two-letter Code		Ratings of samples by Panelists				
Panelist No.	Slight heat	Moderate Heat	Control (5)	Slight Heat (5)	Control (5)	Moderate Heat (10)	Pass
1	94	19	5	5	5	10	Yes
2	62	92	5	4.5	5	10.5	Yes
3	74	53	5	5	5	15	No
4	98	80	5	5	5	10	Yes
5	46	17	5	10	10	15	No
6	78	44	5	1.25	5	9.25	No
7	90	12	5	5	4.6	10	Yes
8	82	37	5	5	5	10	Yes
9	19	88	5	5	5	10	Yes
10	85	66	5	5	5	10	Yes
11	86	79	5	5.2	5	15	No
12	26	14	5	5	5	10	Yes
13	76	42	5	10	5	15	No

Table 29: Panelist results of the diluted extracts prepared by the ASTM method.

	Ratings of samples by Panelists							
Panelist No.	Control	A	Control	B	Control	C	Control	D
1	5	0	5	0.5	5	0.4	5	0.1
2	5	4.5	10	0	5	0	5	0
3	1.25	0	2	0	2	0	1.25	0
4	5	0.1	1.25	0.1	1.25	0.1	1.25	0.1
5	15	0	10	0	5	0	10	0
6	10	1.25	5	1.25	5	0	5	0
7	5	0	5	0	5	0	10	0
8	5	0	5	0	5	0	5	0
Mean	6.41	0.84	5.41	0.19	4.16	0.01	5.31	0.01
Standard deviation	4.20	1.58	3.20	0.45	1.58	0.14	3.32	0.05
SHU calculated	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

In which A is “Amanecer”, B is “Pasión”, C is “Carnaval” and D is “Bonanza”. The average result for all four varieties was zero heat, meaning the Capsaicinoid concentrations are extremely low and the panelist was not able to detect it in the diluted extract.

8. 3. 1 Sensory heat rating ballot for sensory training session

Pungencia de Aji Dulce
Adiestramiento #1

N° de Panelista_____
Fecha _____

Instrucciones: Se le estará proveyendo muestras de diferentes concentraciones de capsaicina con su codificación. Cuando el investigador así lo indique tomar la primera muestra empezando por la izquierda y terminando con la derecha. Evaluar la muestra y use la escala provista para asignar la muestra en el rango que corresponde de pungencia y marque haciendo una línea vertical sobre la escala, siendo "0" nivel cero de pungencia y "15" nivel fuerte de pungencia. Entre cada muestra tomar agua y comer galleta soda para eliminar residuos, este paso será cronometrado por el investigador. Gracias por su cooperación.

Comentarios:_____
_____
_____
_____
_____

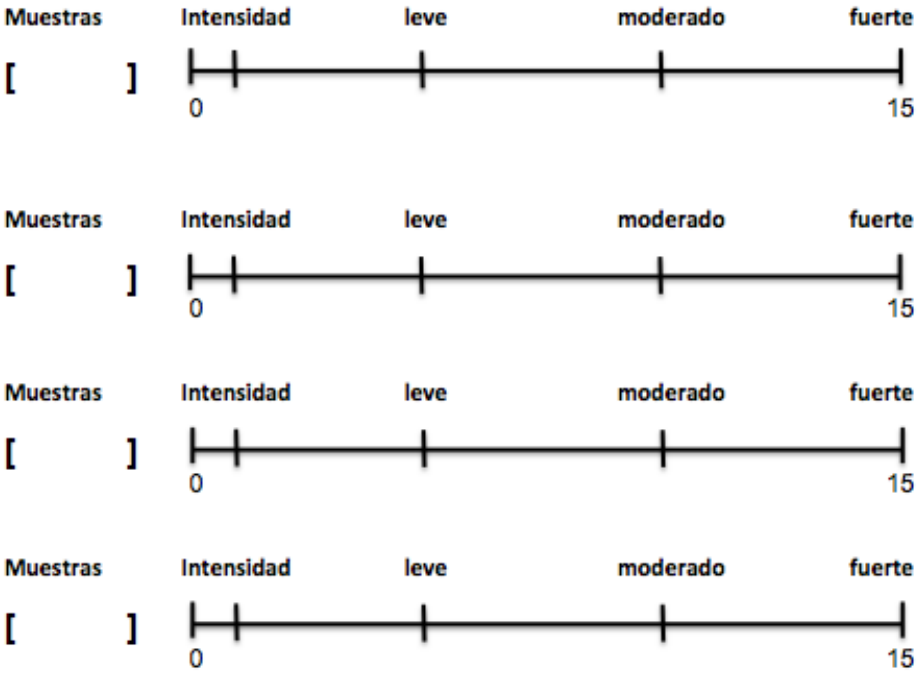
8. 3. 2 Final sensory analysis rating ballot

Hoja de Panelista:

Universidad de Puerto Rico- Recinto Universitario de Mayagüez
CITA-Programa de Ciencia y Tecnología de Alimentos
Prueba Sensorial

N° panelista _____ Muestra: Ajíes dulces (*Capsicum chinense*) Fecha: _____

Instrucciones: Se le están entregando 4 muestras preparadas de Ajíes dulces (*Capsicum chinense*). Favor mantenerlas en el orden en que se le han sido entregadas. Anote los números de las muestras en los espacios provistos abajo. Luego, Enjuáguese antes y entre las muestras con agua y galletas con el fin de limpiar los receptores y eliminar cualquier residuo que quede de la muestra anterior. Espere 90 segundos entre las muestras (que será cronometrado)
Finalmente determine la intensidad de pungencia (picor) del ají dulce que le produce cada muestra y marque haciendo una línea vertical sobre la escala que aparece abajo. Incluya sus comentarios si lo desea.



Comentarios:

_____
_____
_____
_____

8. 3. 3. Exemption letter from the Committee for the Protection of Human Subjects in Research (CPSHI, Comite para la Proteccion de los Seres Humanos en la Investigacion)

Institutional Review Board
CPSHI/IRB 00002053
University of Puerto Rico – Mayagüez Campus
Dean of Academic Affairs
Call Box 9000
Mayagüez, PR 00681-9000

October 29, 2014

Dr. Linda Wessel Beaver
Department of Crops & Agroenvironmental Sciences
College of Agricultural Sciences
University of Puerto Rico at Mayagüez
Call Box 9000
Mayagüez, PR 00681-9018

Dear Dr. Beaver,

As president of the Institutional Review Board (IRB) of the Mayagüez Campus of the University of Puerto Rico I have considered the modified version of the Review Application and accompanying documents for your project titled *Pumpkin and Sweet Chili Pepper for Puerto Rico: Variety Improvement*. (Protocol number 20140417).

On October 28, 2014, you submitted a modification of the protocol. After reviewing all the documentation submitted, this project is still considered exempt under 45 CFR 46.101(b)(2) from 45 CFR 46.101(b)(6) requirements.

Any modifications or amendments to the approved protocol or its methodology must be reviewed and approved by the IRB before they are implemented. The IRB must be informed immediately if an adverse event or unexpected problem arises related to the risk to human subjects, or of any complaint concerning this project. The IRB must likewise be notified immediately if any breach of confidentiality occurs.

We appreciate you commitment to uphold the highest standards of human research protections and remain,

Sincerely,

Aidsa I. Santiago Román, Ph.D.
President
CPSHI/IRB
UPR - RUM

Telephone: (787) 832 - 4040 x 6277, 3807, 3808 – **Fax:** (787) 831-2085 –**Webpage:** www.uprm.edu/cpshi
Email: cpshi@uprm.edu