Cucurbita Flowers as a New Food Product for Puerto Rico:

Quality and Nutritional Assessment

By

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Abstract

Male pumpkin (*Cucurbita* spp.) flowers are potentially an important source of several nutrients, carotenes, ascorbic acid, folic acid, vitamins, and minerals that humans should consume in their daily diet. The aim of this research was to evaluate the physicochemical, nutritional, and visual quality, and carry out sensory evaluations of flowers of locally adapted pumpkin germplasm, thus exploiting a traditional local crop for the development of new high-value products for local consumption as well as for export. Male flowers from four different genotypes of *Cucurbita* produced under organic conditions were harvested from the Lajas Substation of the University of Puerto Rico, Mayagüez Campus: 'Taína Dorada', 'Verde Luz', 'Soler', and 'Bush White Scallop'. Flowers were minimally processed and stored without and with modified atmosphere packaging (MAP) (5% O_2, 11% CO_2, and 84% N_2) at 5 and 10°C for a period of 7 days to evaluate shelf life. Weight loss, visual quality, vitamin C, β-carotene, total soluble solids, pH, total acidity, total phenolic, and DPPH were evaluated before and after storage. At both storage treatments, flowers without and with MAP had a life span of no more than 5 days before presenting an unmarketable appearance. At 5°C, flowers without and with MAP lost up to 14.8% of initial weight. In contrast, at 10°C flowers without MAP lost up to 19% of initial weight and flowers with MAP up to 14%. At both temperatures, Bush White Scallop lost the most weight for both storage treatments. On the 4^{th} and 5^{th} day of storage flower petals were curled moderately to severely in both storage treatments, although flowers with MAP had a slightly better visual quality than flowers without MAP. Verde Luz and Soler had the highest amount of phenolic content (332 and 342 mg GAE/100 g fresh weight [FW]), and antioxidant activity (94.2 and 91.8 µM TEAC/g FW). Bush White Scallop had a good source of beta-carotene content (14.1 mg/100 g FW) compared with all the other genotypes. Ascorbic acid content averaged 16.15 mg/100 g FW with no significant difference

between genotypes. After 5 days of storage at 5°C vitamin C decreased (to 6.03 mg/100 g FW without and 3.83 mg/100 g FW with MAP) and beta-carotene increased (to 13.9 mg/100g FW without and 16.1 mg/100 g FW with MAP). Total acidity decreased to 0.030% without MAP and to 0.020% with MAP while pH increased to 7.52 and 7.36, respectively. Total phenolic compounds decreased to 150.3 mg GAE/100 g FW (without MAP) and 118.4 mg GAE/100 g FW (with MAP). DPPH decreased to 33 µM TEAC/g FW (without and with MAP). Similar results were observed for flowers stored at 10°C. In a sensory evaluation for texture, flavor and general acceptance flowers were rated "liked moderately" on a 9-point hedonic scale. Cooked flowers were also rated as "like moderately." Since flowers have a short shelf life, it is recommended to consume flowers fresh or cooked to ensure good taste and quality. Production of pumpkin flowers for consumption has the potential to contribute added value to Puerto Rico's gastronomy and agricultural economy.

Resumen

Las flores masculinas de calabaza (*Cucurbita* spp.) son consideradas una importante fuente de nutrientes, carotenos, ácido ascórbico, ácido fólico, vitaminas y minerales que los seres humanos deben consumir en su dieta diaria. El objetivo de esta investigación fue evaluar las características nutricionales, físico-químicos, calidad visual y análisis sensorial de flores de distintas variedades de calabazas adaptadas localmente, aprovechando así una siembra tradicional para el desarrollo de nuevos productos de alto valor para consumo local o su exportación. Flores masculinas de una siembra orgánica de cuatro diferentes genotipos de *Cucurbita* fueron cosechadas de la Estación Experimental Agrícola en Lajas de la Universidad de Puerto Rico, Recinto de Mayagüez: 'Taína Dorada', 'Verde Luz', 'Soler' y 'Bush White Scallop'. Estas flores fueron mínimamente procesadas y almacenadas sin y con Empaques de Atmósfera Modificada (EAM) (5% O_2, 11% CO_2 y 84% N_2) a 5 y 10 °C por un periodo de 7 días para evaluar su vida útil. Pérdida de peso, calidad visual, vitamina C, β-caroteno, sólidos solubles, pH, acidez total, fenoles totales y DPPH fueron evaluados antes y después de almacenamiento. En ambos tratamientos de almacenamiento, sin y con EAM, las flores tuvieron una vida útil de no más de 5 días ya que estas presentaron una apariencia visual poco deseada para poder ser mercadeables. A 5°C, flores sin y con EAM perdieron hasta 14.8% de su peso inicial. A 10°C, flores sin EAM perdieron hasta un 19% del peso inicial, mientras que las flores con EAM un 14%. A ambas temperaturas, Bush White Scallop perdió más peso para ambos tratamientos durante el almacenamiento. Del 4to al 5to día de almacenamiento, las flores fueron clasificadas como pétalos enroscados moderadamente a pétalos enroscados severamente para ambos tratamientos. Durante el almacenamiento, las flores con EAM tuvieron una calidad visual ligeramente superior a las flores sin EAM. Verde Luz y Soler tuvieron la mayor cantidad de contenido fenólico (332 y 342 mg GAE/100 g peso fresco [PF]) y actividad

antioxidante (94.2 y 91.8 µM TEAC/g PF). 'Bush White Scallop' tuvo una buena fuente de contenido de beta-caroteno (14,1 mg/100 g PF) en comparación con todos los otros genotipos. El contenido de ácido ascórbico fue un promedio de 16.15 mg/100 g PF, sin diferencias significativas entre los genotipos. Después de 5 días de almacenamiento a 5°C, la cantidad de vitamina C disminuyó (6.03 sin y 3.83 mg/100 g PF con EAM) y β-caroteno aumentó (13.9 mg/100 g PF para las muestras sin EAM y 16.1 mg/100 g PF para muestras con EAM). Acidez total disminuyó hasta 0.030% sin EAM y 0.02% con EAM mientras que pH aumentó hasta 7.52 (sin EAM) y 7.36 (con EAM). El compuesto fenólico total disminuyó hasta 150.3 mg GAE/100 g PF (para muestras sin EAM) y 118.4 mg GAE/100g PF (para muestras con EAM). DPPH disminuyó hasta un promedio de 33 µM TEAC/g PF (sin y con EAM). Resultados similares fueron observados para muestras almacenadas a 10°C. En los análisis sensoriales para textura, sabor y aceptación general, las flores fueron clasificadas como "me gusta moderadamente" en textura, sabor y aceptación general siguiendo la escala hedónica de 9 puntos. Las flores cocidas también fueron calificadas como 'me gusta moderadamente'. Dado que las flores de calabaza tienen una vida útil corta, se recomienda consumir flores frescas o cocidas para asegurar un buen sabor y calidad. Al ver la aceptabilidad de las flores entre los panelistas, estas tienen potencial para aportar un valor añadido a la gastronomía y economía agrícola de Puerto Rico.

Dedication

To God, for giving me the strength I needed to accomplish this difficult task.

To my grandparents, Freddy (may you rest in peace) and Taty, for always telling me to pursue my dreams even if you guys didn't understand all the sacrifices that it implied.

To my father, Edwin, for teaching me to always have a positive mind, that there is no obstacle that I can't overcome and to always shine bright.

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1. Introduction

Cucurbita is the name given to the genus that includes the squash, pumpkins and gourds. Flowers of species in this genus, especially *C. pepo*, are edible and are considered to be rich in vitamin C with high beta-carotene levels (Hosseinzadeh & Amjadi, 2012). The yellow color of the petals is correlated to their levels of carotenoids (Muntan, Muntean, & Duda, 2013).

Many countries consume pumpkin flowers as part of their diet, such as Mexico, Spain, Italy and Thailand (Paris & Janick, 2004). The culinary use of flowers on the day of anthesis may have been practiced by Native Americans before the European encounter. Pumpkin flowers are used in a wide variety of recipes, such as: pumpkin flower soup, "quesadillas," tamales, and desserts (Asiain-Morales, 2008).

The nutritional, physiological, and physicochemical values of pumpkin flowers have been studied. Results obtained from USDA Nutrient Standard Database, (2017) show that raw pumpkin flowers have 0.9 g of fiber/100g. Pumpkin flowers are reported to contain 37 mg of Ca and 0.88 mg of Fe per 100 g compared to Ca and Fe content in cooked pumpkin pulp (11.0 mg and 0.2 mg per 100 g respectively). In addition, flowers contain folic acid in the form of folate in a concentration of 41 μg/100 g.

The production of fruits, vegetables, and flowers is considered to be an important sector in the total world agricultural productivity (Gopinadhan Paliyath, Murr, Handa, & Lurie, 2008). In 2011, 640 million tons of fruit and 1 billion tons of vegetables were gathered throughout the whole world (Food and agriculture organization, 2013). According to the Department of Agriculture of Puerto Rico (Departamento de Agricultura de Puerto Rico, 2015), the farm gate value of tropical pumpkin (*C. moschata*) during 2013-2014 was $20.95 per quintal (100 lbs) with a production of 206,691 quintals of pumpkins. This represents a total value of production of $4.33 million which is an 8.55% of total vegetable production for that year. Although production of pumpkin fruits is an important sector of horticultural production in Puerto Rico, currently, there is no commercial production of harvested pumpkin flowers on the island. Introducing this product to Puerto Rico will benefit its gastronomy, developing a high-value product for local consumption and possible exportation to the rest of the islands in the Caribbean.

An important aspect of using pumpkin flowers is post-harvest treatment. Minimal processing includes treatment of the flower from harvesting until the flower is properly packed for the consumer. Treatment after harvesting involves transportation of flowers from farm to where they are processed, elimination any unwanted parts such as pistil, cleansing, and following correct procedure of packing of flowers. This process helps extend the shelf life of the product while keeping intact its sensorial and organoleptic properties. Moreover, it is important to apply proper techniques that reduce its deterioration after harvesting (Lopez, 2007). Its short shelf life limits the market of pumpkin flowers (Aquino-Bolaños, Urrutia-Hernández, López Del Castillo-Lozano, Chávez-Servia, & Verdalet-Guzmán, 2013).

The long-term goal of this study is to introduce the *Cucurbita* flower as a new food product for Puerto Ricans. More specifically, this research evaluated how locally harvested pumpkin flowers were affected chemically and nutritionally by minimal processing. Additional aspects of this study were to carry out nutritional tests including: vitamins, minerals, carotenoids, and antioxidants. The physicochemical properties of the pumpkin flowers, such as appearance, color, shelf life analysis using modified gas compositions, and physiological loss was also studied. Lastly, sensory tests and surveys of consumer impressions and acceptance were performed.

2. Objectives

2.1. General Objectives

Evaluate the physicochemical and nutritional quality of flowers of locally adapted cultivars of pumpkin.

2.2. Specific Objectives

- Develop protocols for minimal processing and canning of pumpkin flowers.
- Carry out sensory quality tests of fresh and minimally processed pumpkin flowers.

3. Literature Review

3.1. Pumpkin and its Uses

The edible mature fruit of members of the genus *Cucurbita* are known as pumpkins and squashes (Paris, 1986). Pumpkins belong to the Cucurbitaceae family and most the species of this family are used as food. In Puerto Rico, the most common species of *Cucurbita* is *C. moschata*. Its mature fruit is eaten boiled, steamed, in stew or as an ingredient of some desserts. Pumpkin or squash are important foods in several cultures, such as Mexico. These flowers are versatile ingredients. Their acceptance will vary depending on the market culture (Á. Custodio, personal communication).

3.2. Pumpkin Flowers

3.2.1. Botanical Characteristics

The pumpkin plant produces male (Figure 1a) and female flowers (Figure 1b) in the same plant meaning they are monoecious. Male flowers tend to have a smaller peduncle. Male flowers produce pollen that must be transferred to the female flower. Bees are essential in this transferring process (Fornaris, 2009). Female flowers have a small ovary (immature fruit) at their base and this too is edible. In most varieties of tropical pumpkin grown in Puerto Rico, male flowers appear first, followed by female flowers about one to two weeks later. Male flowers usually appear about 50 to 60 days after direct seeding, although this varies with variety and environmental conditions (L. Wessel-Beaver, personal communication).

(a)

(b)

Figure 1: Male (a) and female (b) *Cucurbita pepo* flowers. The female flowers have an ovary (immature fruit) at their base.

3.2.2. Edible Flowers

Although, pumpkin flowers are edible, not all flowers fall into this category. In order to be edible, flowers must have the following characteristics: proper chemical composition, appropriate cultivation procedures (free of non-organic pesticides, herbicides, and fertilizers), and be completely microbiologically innocuous (Lara-Cortés, Osorio-Díaz, Jiménez-Aparicio, & Bautista-Baños, 2013). The use of edible flowers can add flavor, exotic aroma and freshness to a meal (Kou, Turner, & Luo, 2012) while bringing attention to the eye of the consumer.

There are many culinary uses that can be given to edible flowers and these can be served as: fresh, dried, in oil, liquor or honey, macerated, stuffed, roasted or used in different dishes (Anca, Cantor, Buta, & Horţ, 2013). The pumpkin flower is consumed in many countries of Europe and Central America. The species that is used is almost always *C. pepo*. It is a common ingredient in the Mexican diet and it is the only flower that is in high demand all year round (Sotelo, López-García, & Basurto-Peña, 2007). It has a sweet flavor and mild aroma. The flowers are used in various recipes including pumpkin flower soup, "quesadillas," tamales, or stuffed, having great acceptability among consumers (Asiain-Morales, 2008).

3.2.3. Chemical Composition of Flowers

Pumpkin flowers (specifically those of *C. pepo*) are considered to be a rich source of minerals, folic acid, essential amino acids, vitamin B1 and B2 (Sotelo et al., 2007; Talavera, 1999). These are also rich in vitamin C with high beta-carotene levels (Hosseinzadeh & Amjadi, 2012). The USDA National Nutrient Database (2017) for Standard Reference for raw pumpkin flowers, states that these have approximately 49 mg of P, 39 mg of Ca, 173 mg of K, 24 mg of Mg, and 0.70 mg of Fe and Se (Table 1). Flowers have a high moisture content, low protein, fat, ash and carbohydrate content. There is little information on chemical parameters of pumpkin flowers. Table 2 summarizes the chemical parameters on previous studies on *C. pepo* flowers. Pumpkin flowers have an acidic pH, a moderate amount of sugars, and low content of organic acids.

Table 1: Nutrient Data for raw pumpkin (*Cucurbita pepo*) flowers in literature cited

Proximate Analysis	USDA (2014)	Asiain-Morales (2008)	López et al. (2007)
Moisture (%)	95.15	74.28	90.66
Energy (kcal/100 g)	15.00	-	-
Protein (%)	1.03	1.99	1.30
Fat (%)	0.07	-	-
Ash (%)	0.48	0.80	0.80
Dietary fiber (%)	-	-	9.23
Carbohydrate (%)	3.28	-	-
Minerals			
Ca (mg/100 g)	39.00	-	-
Fe (mg/100 g)	0.70	-	-
Mg (mg/100 g)	24.00	-	-
P (mg/100 g)	49.00	-	-
K (mg/100 g)	173.00	-	-
Na (mg/100 g)	5.00	-	-
Se (mg/100 g)	0.70	-	-

Table 2: Physicochemical parameters of *Cucurbita pepo* flowers in literature cited

	Aquino-Bolaños et al. (2013)	Asiain-Morales (2008)
Soluble solids (˚Brix)	4.57	-
Titratable acidity (g citric acid/100g FW)	0.22	-
pH	5.79	-
Color (b* parameter)	-	21.93

FW = Fresh weight of flowers

3.2.4. Antioxidants

Antioxidants play an important role in foods from a health factor point of view (Prakash, Rigelhof, & Miller, 2011). They are known to trap free radicals (Birben et al., 2012). These free radicals are defined as molecules that contain one or more unpaired electrons, giving them reactivity. Antioxidants can be divided into two groups: enzymatic and non-enzymatic. Enzymatic antioxidants are produced by the body, while non-enzymatic antioxidants (vitamin C, beta-carotene, and phenolic compounds) are not produced by the body, but can be obtained in food or supplements.

3.2.4.1. Phenolic Compounds

Compounds like phenolic acids (Figure 2), polyphenols and flavonoids scavenge free radicals, by donating a hydrogen atom, such as peroxide, hydroperoxide, or lipid peroxyl and inhibit any oxidative mechanisms (Kähkönen et al., 1999; Rice-Evans, Miller, & Paganga, 1996), that may lead to degenerative diseases (Prakash, Rigelhof, & Miller, 2011). Flower pigments are considered to be rich in phenolic compounds which include phenolic acids, flavonoids, and anthocyanins (Mlcek & Rop, 2011). Phenolic compounds in ornamental flowers range from 2.53 to 160 mg GAE/g FW. The variations in antioxidant compounds in flowers is due to floral developmental processes and the last steps of senescence. This is attributed to the biochemical and

molecular mechanisms that are activated to counteract the increase of free radicals (Cavaiuolo, Cocetta, & Ferrante, 2013).

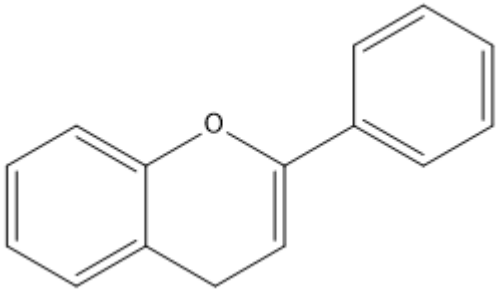


Figure 2: Structure of phenolic acids (Ghasemzadeh & Ghasemzadeh, 2011).

3.2.4.2. Beta-carotene

Approximately 60 different carotenoids occur in plant tissue, many are in the form of β-carotene (Figure 3), α-carotene, and lycopene (Seroczyńska, Korzeniewska, Sztangret-wiśniewska, & Niemirowicz-szczytt, 2006). Carotenoids are known to be singlet oxygen quenchers (Di Mascio, Kaiser, Devasagayam, & Sies, 1991; Sies, 1992) and lipid peroxidation chain breakers (Frei, 1994) (cited in Robaszkiewicz, Bartosz, Ławrynowicz, & Soszyński, 2010). Up till now, there is no official recommended dietary intake of carotenoids but the recommended intake of vitamin A (beta-carotene) is 1-3 mg/day of retinal equivalent (Institute of Medicine, 2001). Pumpkin flowers are a good source of β-carotene. A study by Serocyznksa et al. (2006) demonstrated that fresh male winter squash (*C. maxima*) flowers had beta-carotene content ranging from 1.01 to 13.35 mg/100 g (Table 3). Carotenoid biosynthesis tends to increase during ripening of vegetables that contain any carotene compound (Gross, 1987 cited in Sharma & Rao, 2013).

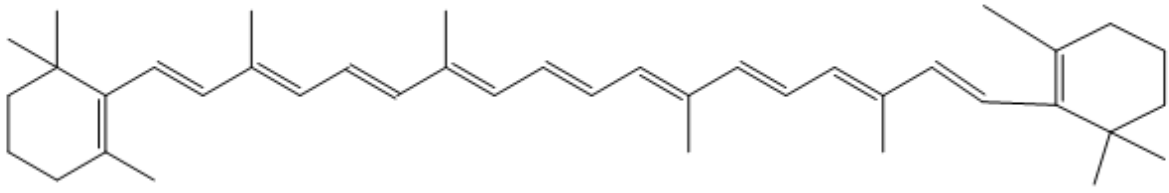


Figure 3: Structure of beta-carotene compound (Kiokias & Oreopoulou, 2006).

3.2.4.3. Ascorbic acid

Ascorbic acid (AA) (Figure 4) is a water soluble vitamin that acts as an antioxidant and reducing agent, protecting from damage caused by free radicals (National Cancer Institute, 2016). This vitamin also participates in collagen synthesis to fight symptoms of scurvy (Smirnoff, 1996). The last step in its pathway is the conversion of L-gulonolactone to L-ascorbic acid, by the enzyme L-gulonolactone oxidase, which is deficient in humans and some animals (Linster & Van Schaftingen, 2007; Smirnoff, 1996). For this reason, AA is an important nutrient in the human diet. The average daily recommended dose of AA in adults is 75 to 90 mg/day (National Institutes of Health, 2011). According to the USDA Nutrient Standard Database, (2017) for raw pumpkin flowers, the content of AA is approximately 28 mg/100 g of FW. Studies by Aquino-Bolaños et al. (2013) showed that *C. pepo* flowers have 16.51 mg/100 g FW of AA content (Table 3). AA concentration can be easily reduced by exposure to heat and oxygen during minimal processing, packaging or storage of foods (Nielsen, 2003).

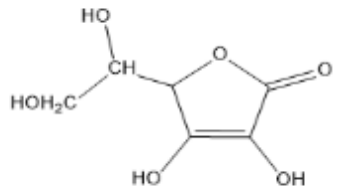


Figure 4: Structure of ascorbic acid compound (Nielsen, 2003).

Table 3: Bioactive compounds in previous studies of *Cucurbita* flowers.

	Aquino-Bolaños et al.(2013)	Loubet González (2015)	Seroczynska et al.(2006)	Tarhan, et al (2007)	Lopez et al. (2007)	Asiain-Morales (2008)
Total phenolic	334.60 mg GAE/100 g FW	8.76 mg GAE/g DW (876 mg GAE/100 g DW)	-	0.152 µg GAE/µg DW (152 mg TEAC/g DW)	-	-
Antioxidant activity	62.00 µmol ACE/100 g FW (0.62 µmol/g FW)	17.96 µmol TEAC/g DW	-	-	-	-
Ascorbic acid	16.51 mg/100 g FW	-	-	-	12.00 mg/100 g FW	4.79 mg/100 g FW
β-carotene	-	1334.77 µg/g DW (133.47 mg/100 g DW)	1.01-13.35 mg/100 g FW	-	-	-

FW = Fresh Weight of Flowers; DW = Dry Weight of flowers; GAE = Gallic acid equivalent; Ac = Ascorbic acid equivalent; TEAC = Trolox equivalent. Values in parenthesis were converted to same units for better comparison of data.

3.2.4.4. Analytical Methods

3.2.4.4.1. DPPH (1,1-Diphenyl-2-picryl-hydrazyl) Method

DPPH (

Figure ***5***) is an organic stable free nitrogen radical that accepts a hydrogen atom from a donor of the extract samples, making its solutions lose its purple color (Tirzitis & Bartosz, 2010). The mechanism that occurs is as follows: the decrease in absorbance of DPPH· is monitored at a wavelength of 515 to 517 nm (Figure 6). When DPPH· is reduced by an antioxidant molecule (AH) or reactive specie (R·) the absorption disappears producing colorless solutions (Brand-Williams, Cuvelier, & Berset, 1995). DPPH is an electron transfer (ET) based reaction that has no similarity to the peroxyl radicals involved in lipid peroxidation. Peroxyl radicals are normally involved in hydrogen atom transfer (HAT) reactions. This may limit the use of DPPH method since antioxidants that react quickly with peroxyl radicals will most likely react slowly to DPPH (Huang, Prior, & Ou, 2005).

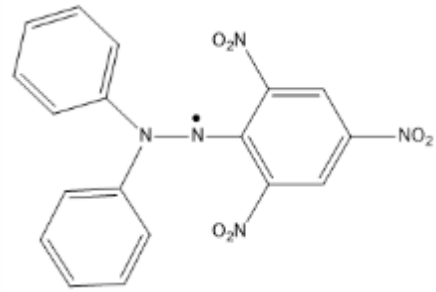


Figure 5: DPPH Structure (Huang et al., 2005) .

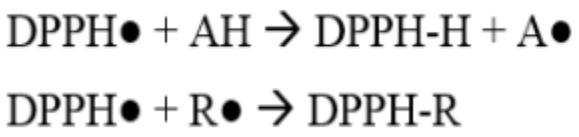


Figure 6: Mechanism demonstrating reduction of DPPH (Brand-Williams et al., 1995).

3.2.4.4.2. ORAC Assay

The Oxygen Radical Absorbance Capacity (ORAC) method is a hydrogen atom transfer (HAT) based reaction, which monitors competitive reaction kinetics and the quantification is derived from the kinetic curves. This type of assay is composed of the following components: an azo radical initiator 2,2-Azobis (2-amidinopropane) dihydrochloride (AAPH), a molecular probe (fluorescent reader), antioxidant, and reaction kinetic parameters for antioxidant quantification. Fluorescein (FL) is going to be consumed as the reaction progresses, while at the same time, decreasing its intensity. FL is inhibited in presence of an antioxidant and the data is calculated by the area under the curve (AUC) (Huang et al., 2005). This method evaluates the values as Trolox equivalents (Haytowitz & Bhagwat, 2010). This assay provides a direct measure of lipophilic and hydrophilic chain-breaking antioxidant capacity versus the peroxyl radicals (Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002).

3.2.4.4.3. Total Phenolic Assay

Total phenolics can be measured using the Folin-Ciocalteau (F-C) assay. The F-C assay (tungsten and molybdenum) is a colorimetric method that consists of an oxidation reaction of phenols by using alkali sodium carbonate, which yields a concentration of phenolate ions. The phenolates reduces the yellow F-C reagent switching it into a blue color, which is spectrophotometrically measured at 765 nm (Singleton & Rossi, (1965) cited in Cicco & Lattanzio, 2011). The intensity of absorption at 765 nm is proportional to the phenolic concentration (Waterhouse, 2002). This assay normally expresses the number of phenolic compounds in Gallic

acid equivalents. Gallic acid is found in the form of acids, esters, catechin derivatives, and hydrolysable tannins among various plant materials. It is one of the most biologically active phenolic compound of plant origin and that is why it is used to determine antioxidant activity in plant extracts (Karamaæ, Kosiñska, & Pegg, 2006).

3.2.4.4.4. Ascorbic Acid Assay

Commonly, AA content is determined by the 2,6-dichloro-indophenol method (AOAC 967.21, 2010). The mechanism (

Figure *7*) of this process is that L-ascorbic acid (colorless) is oxidized to L-dehydroascorbic acid (brown) by 2,6-dichloroindophenol which is an oxidation-reduction indicator. At the endpoint of titration, excess unreduced dye appears to have a rose-pink color in acid solution. L-ascorbic acid is likely to be damaged by oxidative deterioration. Therefore, the procedure should be performed at low pH and in the presence of a chelating agent to reduce the possibility of oxidation (Nielsen, 2003).

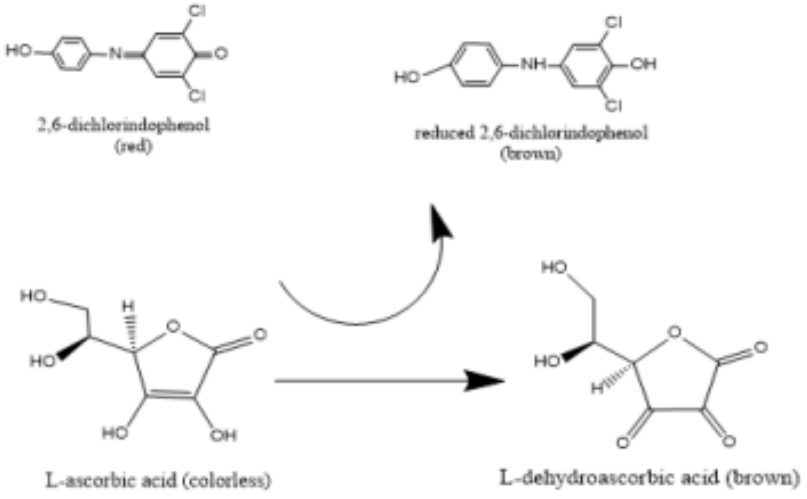


Figure 7: Chemical reaction between L-ascorbic acid with the indicator 2,6-dichloroindophenol indicator (Nielsen, 2003).

3.2.5. Minimal Processing

As stated by the International Fresh-Cut Produce Association (IFPA, 1999), products minimally processed can be described as "any fruit or vegetable, or any combination, which has been physically altered from its original form, but has remained in its fresh condition" (cited in De Oliviera, Rocha, Wurlitzer, De Jesus, & Mangan, 2012). Processing foods lead to a quicker physiological deterioration, biochemical changes, and microbial degradation of the product (O'Brien & Francis, 2003). If the product is subjected to peeling, cutting or shredding during minimal processing, fluids of the plants cells are released causing browning and microbial growth (Tripathi, Gupta, & Kumar, 2011). New techniques for maintaining quality and inhibiting any undesirable microbial growth such as usage of disinfectants and modified atmosphere packaging (MAP) are requested in all the steps of production (Allende, McEvoy, Luo, Artes, & Wang, 2006; Allende, Tomás-Barberán, & Gil, 2006).

3.2.5.1. Quality Material

Food must be visually appealing for customers to consume it. Edible flowers have the potential to add value to the product to which it is added and increase its price (Kelley, Cameron, Biernbaum, & Poff, 2003). High quality flowers are those that have good appearance in terms of: size, shape, color, and freshness. It is recommended to grow flowers organically (Anca et al., 2013). They must be clean, and free of microbial compounds to ensure a good quality product. The presence of dirt, insects or pathogens on the surface of the flower reduces its quality. Physical characteristics, like crispness and roughness are counted as a quality parameter in selecting good edible flowers (Salunkhe, Bhat, & Desai, 1990). Information about quality of edible flowers will help producers and marketers to create different mixes, package sizes, and pricing techniques that can help increase profits and market share (Kelley, Behe, Biernbaum, & Poff, 2001).

3.2.5.2. Cutting

The majority of flowers should be cut at a stage that permits subsequent floral development and prolonged shelf life (Hardenburg, Watada, & Yi, 1988). Flowers should be harvested at dawn, to avoid high temperatures since this affects quality of the edible flowers until the end of its use (Anca et al., 2013). For cutting of ornamental flowers, sharp tools are used to cut the stems of the mother plant. The angle of the cutting should be in a slanting position and it is important to not crush the stem while cutting (Chandra, Pathak, Rao, & Rajeevan, 2014). Squash blossom cutting, is mostly carried out using pruning shears or sharp knives when flower petals are completely open (after anthesis) and usually 1 cm of the stem if left (University of California Cooperative Extension, 2014). Correct cutting for all fruits and vegetables is vital since every step of minimal processing can affect the quality and microflora of the produce (Allende, Tomás-Barberán, et al., 2006).

3.2.5.3. Transportation

Commercial and edible flowers should be delivered to their destination as quickly as possible after harvesting, due to their high perishability rate. For short distances, flowers are usually transported under refrigeration from the grower to the consumer (Chandra et al., 2014). After harvesting, flowers can be layered between damp towels and placed loosely in plastic bags, refrigerated and transported to a work area (Gegner, 2004). In a study by Villalta et al. (2004), pumpkin flowers were harvested, placed into hinged, polystyrene containers, with three blossom per container and quickly transported to laboratory. The University of Kentucky Cooperative Extension Services, (2012) recommends that when handling edible products, extra time and care is needed to transport the produce from the farm to its destination.

3.2.5.4. Washing and Disinfection

Edible flowers have to be gently washed, and checked for insects and soil residues that can be present on the surface of the plant (Falconnier, 2006). Before gently washing flowers, it is recommended to test one flower for colorfastness since these tend to discolor in water (Rindels, 1995). Flowers that are free of dirt and insects, are ready to eat. If there is any presence of dirt or insects, these can be removed by gently applying a paint or make-up brush. Flowers can also be dipped in room temperature water to remove debris, then placed on a paper towel to dry. Flowers can be gently rinsed in a pan of cool water and stored in iced water in the refrigerator. These can last up to 1 or 2 days (University of California Cooperative Extension, 2014). One must be careful when applying these methods because edible flowers usually have delicate petals. Using these methods results in less damage of flowers in comparison with washing them under a stream of water (Kelley, 2002).

3.2.5.5. Packaging

The objective of packaging is to protect the product from contamination or spoilage while trying to extend its shelf life and providing an added value product to consumers (Bhat, 2013). The function of a package is to protect produce during handling, unloading and transport, absorb moisture or water loss, and permit air flow through ventilation holes (Schuur, 1988). Packing materials should be made of food-grade products to avoid any formation of toxic compounds inside the package and onto the produce (Hardenburg et al., 2004).

Proper packaging should be used to ensure protection of delicate edible flowers. These can be stored in rigid plastic containers similar to those used for storing strawberries and other perishable items (Kelley et al., 2001). Flowers are also stored in polyethylene terephthalate materials and cardboard boxes when they are being exported (Pomario, 2008). These can be packed as clusters in tray packs (Villalta, Ergun, Berry, Shaw, & Sargent, 2004). These packing methods are used with the purpose of extending the products shelf life.

3.2.5.6. Storage Temperature

Temperature may be one of the most important environmental factors when extending fruit, vegetable and herb shelf life (Watada & Qi, 1999) since it helps maintain good quality by reducing metabolic changes, slows down senescence and deterioration of flowers (Watada & Qi, 1999; Hardenburg et al., 1988 cited in Lopez, 2007). For this reason, it is desirable to use low temperatures to preserve fresh produce (De Oliviera et al., 2012). Refrigeration will likely extend the shelf life of most edible flowers but some could be sensitive to chilling injury. Many edible flowers such as viola (*Viola tricolor*), pansy (*V. x wittrockiana*), and nasturtium (*Tropaeolum majus*) can be stored from -2.5 to 2.5°C for up to 2 weeks or from 2.5 to 10°C for one week, while still having good visual quality. On the contrary, edible flowers like scarlet runner bean (*Phaseolus coccineus*) can be stored for one week from 0 to 10°C with good visual quality (Kelley et al., 2003).

3.2.5.6. Post-Harvest Processes

Fresh products should be harvested at their optimum maturity stage since their shelf life is reduced if it is insufficient or excessive (Hardenburg, Watada, & Yi, 1988). All fresh horticulture crops have high moisture content which makes them susceptible to dehydration (wilting and shriveling) and mechanical damage. High moisture content also makes these products susceptible to bacteria and fungus attacks (Kader, 1992). Storing at low temperatures helps retard: "(1) aging caused by maturation, softening or changes in texture and color, (2) undesirable metabolic mechanisms, and production of heat due to respiration rate, (3) loss of moisture and subsequent wilting, (4) decomposition caused by the increase in yeast and fungus" (Hardenburg et al., 1988).

3.2.5.6.1. Transpiration or Water Loss

One of the main causes of deterioration in plants is water loss because it results in loss of salable weight, losses in appearance (shriveling and wilting), poor textural quality (softening,

limpness, loss of crispness and juiciness) and loss of nutritional quality (Kader, 1992). External factors (temperature, relative humidity, and atmospheric pressure) and internal factors (morphological and anatomical characteristics, surface injury and maturity stage) are what most affect transpiration rates (evaporation of water from the plant tissue). Being a physical process, transpiration rate can be controlled by applying treatments such as: coating, wrapping with plastic films, or maintaining high relative humidity and air circulation.

3.2.5.6.2. Ethylene Production

Ethylene is a simple organic compound that affects physiological processes in plants (Kader, 1992). It is a natural product of a plant's metabolism and it is produced by all tissues of higher plants and microorganisms. This compound regulates many processes during growth, maturation and senescence. Ethylene plays an important role in the senescence of cut flowers, but ethylene sensitivity varies depending on the species (Redman, Dole, Maness, & Anderson, 2002). As stated by Have & Woltering, (1997) ethylene binds to a specific receptor forming a complex that starts the process of ripening. Ethylene is first produced in the flower pistil, followed by the petals, inducing the expression of 1-aminocyclonepropane-1-carboxylate (ACC synthase), cysteine proteinase and oxidase. This induction results in an auto-catalytic ethylene production of petals followed by the wilting of flowers (Chandra et al., 2014; Satoh, Shibuya, Waki, & Yusuke, 2005; Teixeira da Silva, 2003). There is no consistent relationship between ethylene production of a given commodity and its perishability. However, exposure of most commodities to ethylene accelerates their senescence. In fresh commodities, ethylene can be reduced by storage at low temperature, by reduced O_2 (less than 8%) and elevated levels of CO_2 (more than 2%) around the commodity (Kader, 1992).

3.2.5.6.3. Senescence

Senescence and death are known to be an integral part of a plant's lifecycle and it is an active process where nutrients are broken down and metabolized from senescing organs to active

growing ones (Teixeira, 2016). This is a natural process that is thought to be correlated to changes in hormones such as the production of ethylene and abscisic acid in flower tissues (Tripathi & Tuteja, 2007). Petals of cut flowers that undergo senescence experience falls in protein content, increase in protease activity, reduction in lipid fluidity, and increase in respiration rates (van Doorn & Stead, 1997). Flowers that are exposed to senescing tend to exhibit a climacteric-like rise in ethylene production (Beyer, 1977; Mayak, Vaadia, & Dilley, 1977; Trippi & Paulin, 1984) producing in-rolling of petals, triggering ethylene synthesis (Nichols, 1968) and causing chemical and physical changes in the microsomal membrane of lipids (Thompson, Mayak, Shinitzky, & Halevy, 1982) of senescing petals (cited in Bartoli, Simontacchi, Montaldi, & Puntarulo, 1996; Davies, 1995; Gopinadhan Paliyath et al., 2008; Teixeira da Silva, 2003).

3.2.5.6.4. Respiration Rate

Respiration rate can be defined as the rate of breakdown of organic materials (proteins, fat, and carbohydrates), into simple end products with the release of energy (Kader, 1992). During this process, oxygen is used and carbon dioxide is produced, releasing heat energy that affects postharvest technology. The loss of food reserves in commodities during respiration causes: acceleration in senescence, reduction in nutritional value of produce, loss of taste, and weight loss (Kader et al., 1989). The rate of deterioration of perishable commodities is directly proportional to the rate of respiration. Cut flowers are highly perishable at 5°C, with a respiration rate ranging from 40 to 60 mg CO_2/kg-hr (Kader, 1992). Studies on the respiration rates of narcissus (*Narcissus tazetta*) flowers demonstrated an increase of the respiration rate with an increase of temperature ranging from 0 to 12.5°C. Hence, respiration rate is related to temperature storage. For this reason, respiration measurements can give an accurate prediction of the shelf life of stored flowers (Cevallos & Reid, 2000).

3.2.5.7. Canning Protocol

During preservation by canning, food is sterilized and sealed in airtight containers to preserve them. The purpose of this process is to retain nutrients and optimum quality of foods, thus preserving fruits and vegetables when at their peak of freshness (Hendren, Burney, & Morris, 2008). The first step in canning is to sterilize jars by washing them with hot water (Safefood 360, 2014). Then raw fruits or vegetables are washed with a chlorine concentration of no more than 200 ppm for one minute to achieve desired sanitizing effect. It is important to re-wash the produce with potable water after the chlorine treatment to eliminate any potential risk of chlorine residues (McGlynn, 2004).This is followed by the blanching process. Blanching can be carried out by immersion in boiling water, hot air or steam, although boiling water is most commonly used (Amin & Lee, 2005; Henry & Massey, 2001). This technique inactivates or kills enzymes, thus aiding in the preservation of the produce. Nutrient retention during this process differs depending on what method and type of vegetable used (Henry & Massey, 2001). After blanching, the produce is thermally shocked or cooled by placing the recently blanched produce in a bath of water and ice. The next step is the addition of preservatives that help to extend the shelf life of the product. There are two types of food preservatives: direct and indirect food additives. Direct food additives are those which are added to a food for a specific purpose. Indirect food additives are those that become part of the food due to its packaging, storage or other handling (FAO, IFIC, & USDA, 2010; Meador & Liting, 2011). An example of a direct food additive is salt, while an example of an indirect food additive is citric acid. Salt is usually added at a specific concentration with water to create a solution. The hot saline solution is then added to the food product. Normal salt solutions can vary from 2-3 % of salt. It would be ideal that the concentration of salt utilized does not affect the taste of the food, in this case pumpkin flowers. On the other hand, the addition of citric acid is considered a crucial step in the process of canning. Citric acid works as an acidity regulator. Its main purpose is to maintain pH of the canned product under 4.6 which is the level recommended by the USDA, (2016) and Codex Alimentarius, (2011). The final step in canning is absolute sealing of the product, labeling and appropriate storage from 0 to 4°C to avoid or retard growth of bacteria that cause spoilage (USDA, 2016).

3.2.5.8. Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) of fresh fruits and vegetables describes the sealing of active respiring commodities in a polymeric film that adjusts the CO_2 and O_2 levels in the package atmosphere. The purpose of this technique is to reduce the levels of O_2 and increase the levels of CO_2 with the objective to control fresh produce metabolism, increasing the shelf life of the packed product (Pasha, Saeed, Tauseef, Rafiq, & Rohi, 2014). Low concentrations of O_2 and high concentrations of CO_2 can reduce the incidence of physiological disorders like those induced by acetylene and chilling injuries (Kader, Zagory, & Kerbel, 1989). MAP replaces the air in the package with a specified gas mixture. Once the gas mixture is injected, no further regulation of the gas composition is applied and the composition will inevitably change. The new conditions in the package will reduce respiration rate while at the same time delay senescence (Kader et al., 1989; Tripathi et al., 2011).

It has been shown that in an atmosphere of 10-15 % CO_2, the quality and nutritional values throughout the storage period for pumpkin (*C. pepo*) flowers is maintained (Aquino-Bolaños et al., 2013). Edible carnations (*Dianthus caryophyllus L.)* and snapdragons (*Antirrhinum majus L*.) stored under MAP had reduced weight loss and good overall visual quality (Kou et al., 2012). There are limited number of studies of storage of edible flowers under MAP because it is difficult to control optimum levels of CO_2 and O_2 for extending shelf life of flowers (Halvey & Mayak, 1989 cited in Vergano & Pertuit, 1993). Storing flowers under MAP, in many cases, has failed to show benefits because the absence of a valid control has contradicted the credibility of results (Reid & Jiang, 2012). If optimum CO_2 levels are exceeded or O_2 levels decrease, injury will occur, causing ripening, physiological disorders, and increase decay. Not all plant material benefit from using MAP. Plants that do not benefit tend to respond differently to the atmosphere generated (Kader et al., 1989).

3.2.6. Physicochemical Characteristics

3.2.6.1. Total Soluble Solids

Sugars and other dissolved solids in solution are referred to as total soluble solids (TTS). In early 19th century a hydrometer scale was developed to directly measure TSS (Ball, 2006). The measurements are evaluated following the degrees Brix scale, where the scale is numerically equal to the percent of TSS. A hydrometer (that measures density) or refractometer (that measures the concentration of a solution by its refractive index) can be used to measure TSS at a specific temperature of 20°C. For example, a solution that is 25°Brix means, that it has 25 g of sugar (and other solutes) and 75 g of water per 100 g of solution. Brix measurements are evaluated in food industries to estimate product sweetness and as a quality control point since sugar content influences cost and marketing of soft drinks (Mettler-Toledo, 2013). The method of refractometry has been used to determine TSS present mostly in fruits and products of fruits. However, it is also used in dairy products, eggs, beers, and vinegar (Cecchi, 2003).

3.2.6.2. Total Acidity and pH

The organic acids present in foods influence the flavor, odor, color, stability and maintenance of quality (Cecchi, 2003). Total acidity is a measure of the amount of acid that there is in a food product. The higher the acidity in food produce, the less susceptible it is to spoilage by microorganisms (Mettler-Toledo, 2012). This quantity is determined by titration of intrinsic acids with a standard base. Total acidity is calculated by the amount of base utilized, concentration of the base used to titrate and weight of sample (Nielsen, 2003). It is necessary to know the major acid present (citric acid, acetic acid, lactic acid, tartaric acid, malonic acid, etc.) in the food product before selecting the conversion factor, since values are expressed as g acid present/100 g of sample (Fellows, Axtell, & Dillon, 1995).

The pH scale is used to determine the acidity or alkalinity of any solution. Measuring pH is important during food processing since it helps to avoid the growth of microorganisms (Queeney, Ken & Mettler, 2007). Also, it helps to determine enzymatic activity, texture of gelatin

and jams, retention of taste and odor of fruit products, stability of artificial colorants in fruit products, verification of the stage of maturity of fruits and selection of packages (Cecchi, 2003). Foods with a pH lower than 4.6 are classified as low acid foods. On the other hand, foods with higher pH can be lowered by adding acid ingredients to avoid the growth of certain toxic pathogens (Cornell University, 2009).Variations in pH can impact the flavor, consistency and shelf life of a food product (Queeney, Ken & Mettler, 2007).

3.2.6.3. Color

The food industry uses color measurements to ensure that a good color quality product is going to the consumer. These measurements are used for the development of new food products or bettering the version of existing food products. Color measurements may also be used for the estimation of pigments (Joshi & Brimelow, 2002). There are several commercially available colorimeters that measure color following the Commission of Illumination (CIE) system or the Hunter scale. These systems define color in a tridimensional space using the coordinates L^*, a^*, and b^*. The lightness coefficient (L^*) ranges from black = 0 to white = 100. The coordinates (a* and b^*) are located on a rectangular-coordinate grid perpendicular to L^* axis. On the horizontal axis of the grid, positive a^* indicates a hue of red-purple while negative a^* indicates bluish-green. On the vertical axis, positive b^* is yellow and negative b^* is blue (Mcguire, 1992a). In order to interpret these values in manner that relates to the way the human eye perceives color, values of a* and b* are converted to hue angle (type of color such as yellow, yellow-orange, etc.) and chroma (color saturation or intensity) (Hunter, 1942; Little, 1975; cited in Mcguire, 1992a).

3.2.7. Proximal Analysis

The categories of proximate analysis are: moisture, ash, crude protein, fat and crude fiber. These analyses were developed to give a broad classification of food components (Greenfield & Southgate, 2003). Moisture is a measure of the amount of water that is found in a material at any given time and is expressed as the percentage of mass of the material that is contributed by the mass of contained water (Nielsen, 2003). Ash is defined as the residue on ignition (ash) using an

incinerator and a muffle furnace. Ash is considered to be the inorganic residue of a material, (expressed as a percentage of the original weight), which remains oxidized after incineration (Nollet & Toldra, 2015). The Kjeldahl method that is used to determine total nitrogen percentage and then protein. Fat was defined as the lipid material that is extracted from food samples by a set of lipid-solubilizing solvents. This is defined as the sum of all fatty acid present in all types of foods (Schmidl & Labuza, 2000).

3.2.8. Sensory Analysis and Visual Quality

Sensory evaluations consist of a set of techniques used to measure human responses to foods while minimizing the effects of brand identity and other aspects that can influence consumer perception. The aim of these analyses is to isolate sensory properties of foods and provide information to product developers, food scientists, and managers about the sensory characteristics of their products (Lawless & Heymann, 2010).

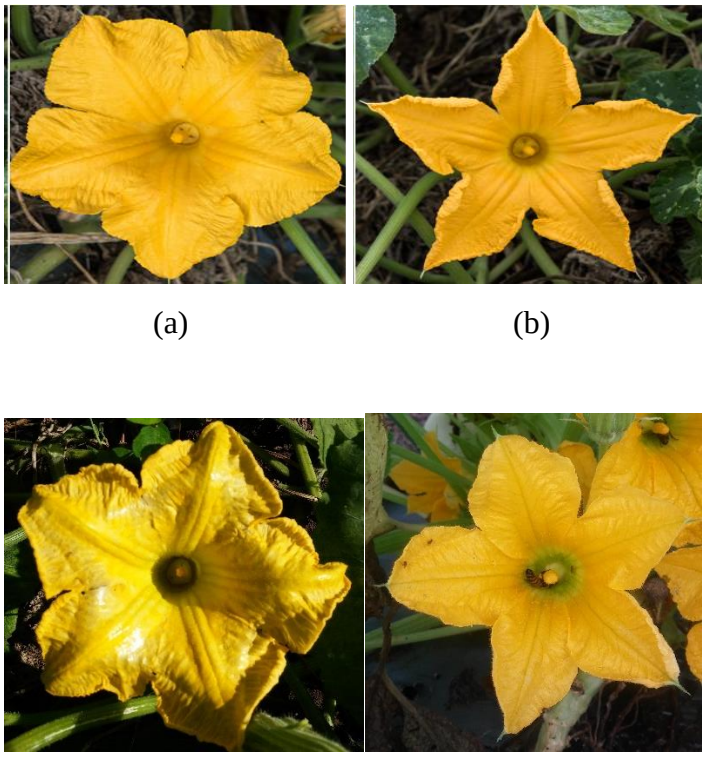
The taste of flowers is sensed differently depending on the receiver's point of view. Most edible flowers have a sweet taste and this is due to the sucrose content (Le Roy et al., 2007). During senescence, the content of sucrose may decrease due to an increase in hydrolysis of fructans. This process is coded as an increase in the enzymatic activity of fructan-1-exohydrolase and a decrease in the activity of sucrose 1-fructosyl transferase in flowers. Changes in taste and texture of flowers are dependent on the type of species; some edible flowers are very tender and crisp while others tend to be more fragile and silky (Mlcek & Rop, 2011). Appearance, size, shape, taste, aroma and coloring are the most important criteria of quality of edible flowers perceived by human senses.

Visual quality or appearance is an important key in the quality assessment of food products. This plays a role in the decisions made by the consumers. In most cases, the evaluations are carried out by a panel of experts, who by visual inspection, analyze samples of the product (Pereira, Reis, & Saraiva, 2009). Visual quality and sensory evaluations can be rated following the 9-point hedonic scale, where 9 = like extremely and 1 = dislike extremely (Meilgaard & Muller, 1987).

4. Methodology

4.1. Source and Minimal Processing of Flower Samples

Three genotypes of *C. moschata* ('Taina Dorada', 'Verde Luz' and E1101-1) and two genotypes of *C. pepo* ('Bush White Scallop' and 'Early Prolific Straightneck') were evaluated for yield of male flowers in the field (Lajas Substation and/or Alzamora Farm of UPRM). Males flowers (Figure 8) were harvested daily, for 30 days for yield performance. For physicochemical, nutritional, and shelf life analysis, three genotypes of *C. moschata* ('Taina Dorada', 'Verde Luz', and 'Soler') and one genotype of *C. pepo* ('Bush White Scallop') were evaluated. For shelf life, male flowers were harvested once or twice a week until sufficient flowers were obtained. The study of characterization and shelf life of male pumpkin flowers lasted from May 2015 until December 2016.



(a) (b)

(c) (d)

Figure 8: Four genotypes of *Cucurbita* flowers (a) Taína Dorada, (b) Verde Luz, (c) Soler and (d) Bush White Scallop. (Photos: Kenneth Mercado y Kathina Toro)

Flower stamens (the pollen producing part of the male flower) were removed, and petals were cleaned with a damp towel using potable water. Once cleaned, 3 to 4 flowers were placed in plastic black trays (136.5 x 177.8 x 35mm) with water absorbent pads and sealed with plastic using a KOCH packing machine (KOCH Ultra Source LLC, Kansas City, MO, U.S.A). Flower samples were sealed without and with MAP (11 % CO_2, 5 % O_2 and 84 % N_2). Trays were placed in a refrigerator (Fogel Caribbean Corp., Aguadilla, PR.) at 5 ˚C and 10˚C at a relative humidity of 77 ± 2 %. After 7 days of storage, physicochemical measurements were evaluated. It should be noted that the measurement of weight loss was done in a separate experiment from the measurement other physicochemical parameters (appearance, CO_2, O_2, Brix, titratable acidity, pH, color, ascorbic acid, beta-carotene, total phenolic content, and antioxidant activity). Proximal analysis, and micronutrients was done on fresh flowers. Sensory panels were used to evaluate fresh and cooked flowers (

Figure ***9***).

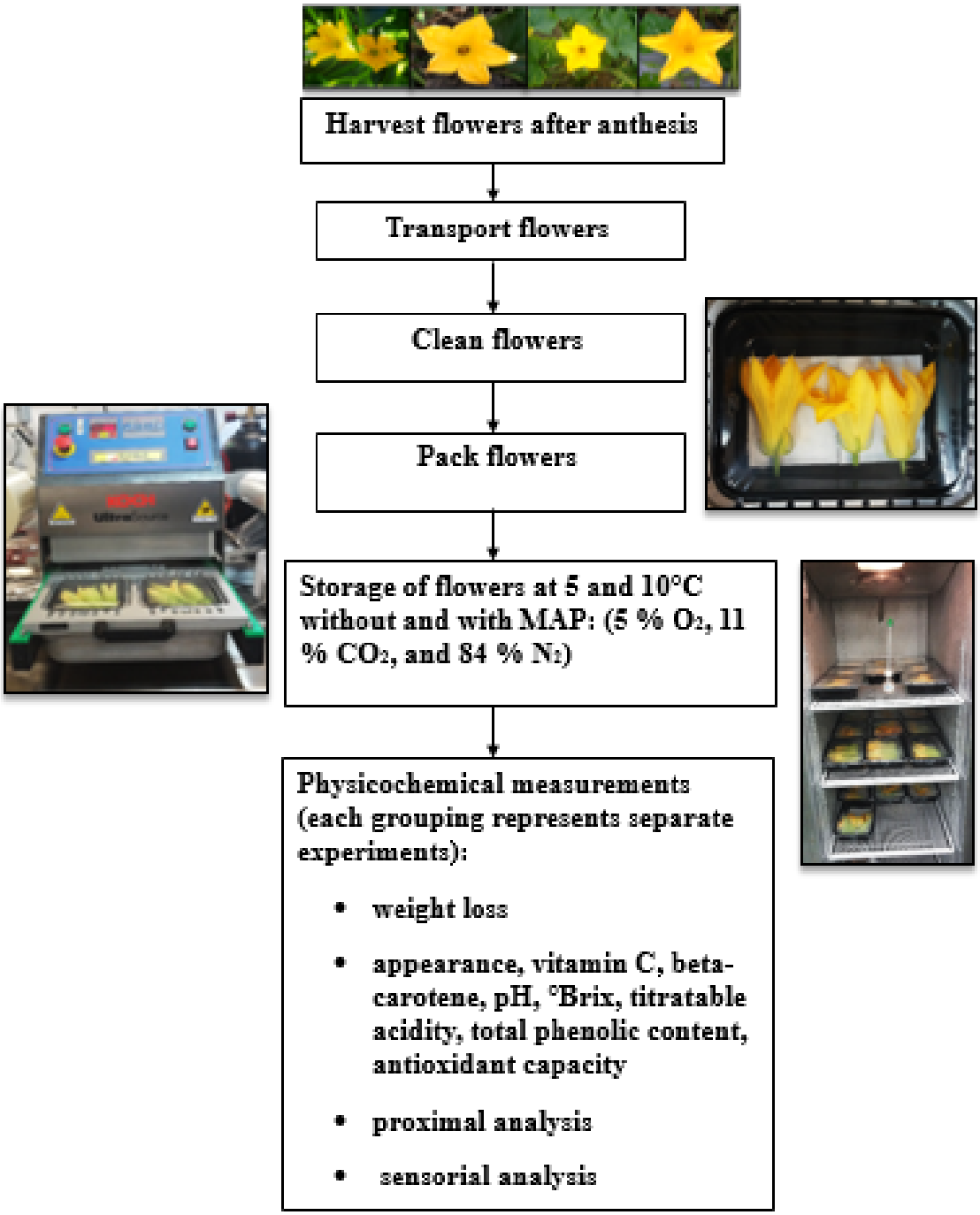


Figure 9: Flow chart of minimal processing of pumpkin flowers.

4.2. Physicochemical parameters measurements

4.2.1. Yield Performance

The five genotypes were divided in the field into two plots where each genotype had four plants per plot. Male flowers that were at anthesis (open flower) were harvested in the field at 7 am and cut from the mother plant using scissors, leaving approximately 5 to 8 cm of the stem. Flowers were counted to determine flower production per plant and per genotype. Harvested flowers were transported in a small isoform refrigerator (305 x 300 x 276 mm) to the Pilot Plant of the Food Science and Technology Program at UPRM. At the Pilot Plant, stems were cut leaving 1 cm of the stem, and flowers were individually weighed and their length was measured. Flowers were weighed using an analytical balance ± 0.01g (Metter PC 16 Instrument Corp., Hightstown, NJ, USA) and a ruler was used for measuring length. Female flowers were harvested on the day of anthesis, and counted to evaluate yield of flowers per genotype. This study was carried out during 30 days from May to June 2015.

4.2.2. Weight loss

Weight loss was measured every other day for approximately 7 days. The sample weight was evaluated using an analytical balance ± 0.01g and following the equation stated by Lucera, Simsek, Conte, & Nobile, (2012).

Equation 1:

$$\%WL\ (t) = \frac{Wo - Wt}{Wo} \times 100$$

Where, % WL (t) = weight loss at time t; W_o = initial sample weight; W_t = sample weight at time t.

4.2.3. Visual Quality

Representative packages of flowers were photographed for both storage temperatures and treatments at days 0, 2, 3, 4, 5, 6, and 7. Later, these photographs were used to rate the flowers on a 5-point scale where 5 = no defects, 4 = petal curled slightly, 3 = petals curled moderately, 2 = petals curled severely, and 1 = flowers wilted and rotten.

4.2.4. CO_2 and O_2 Analysis

Pumpkin flowers were placed in plastic black trays (136.5 mm x 177.8 x 35 mm) with absorbent pads that were sealed without and with MAP (11 % CO_2, 5 % O_2 and 84 % N_2) using a KOCH packing machine. These were observed every other day for up to 7 days to evaluate gas percentage, and flower appearance. Gas analysis of flowers was carried out through a gas analyzer ® (Servomex Company Inc., Brighton, East Sussex, United Kingdom) which measured the percentage of CO_2 and O_2 of each sealed package (Figure 10). Three runs on different days with 9 trays per genotype were carried out to evaluate gas composition of flowers during storage at 5 and 10°C.

(a) (b) (c)

Figure 10: (a) Tray with absorbent pad, (b) Gas Analyzer, (c) Packing equipment

4.2.5. Total Soluble Solids

TSS were evaluated following the AOAC 932.12, (1998) standard method. Four grams of fresh and stored flowers of each genotype were homogenized in a blender (Waring Commercial, Torrington, CT) and a drop of these samples was placed in a digital refractometer (Pal-1, Atago, Toyko, Japan). Values were expressed as °Brix, where 1°Brix equals 1 g of sucrose/100 g of solution (Ball, 2006).

4.2.6. Total Acidity and pH

Total acidity was measured following the AOAC 942.15 (2005) standard method. Four grams of pumpkin flower of each genotype were homogenized with 36 ml of distilled water and filtered at vacuum with Whatman paper No. 1. Titration was carried out with 0.1 N NaOH until a pH of 8.20 was reached. Values were expressed as percentages of citric acid (g citric acid/100 g FW) (Aquino-Bolaños et al., 2013).

Measurements of pH were carried out following the AOAC 942.15, (2005) standard method using a potentiometer (SympHony, SB70P VWR, Cornealius, OR) and a calibrated electrode (AR15, Fisher Scientific). The electrode was calibrated with buffer solutions of 4, 7 and 10 and was directly immersed into the sample solution.

4.2.7. Color

Color (L*, a*, b*) was measured in pumpkin flowers on the outer part of the petal, in the middle position of the petal using a colorimeter (Hunter Associates Laboratory, Inc. Reston, Virginia, USA) calibrated with black and white porcelain tiles. Color was measured in fresh and stored flowers to observe decay in pigment concentration. One petal of each flower was placed on the colorimeter port. Hue angle and chroma were determined using the following formulas (Mcguire, 1992b):

Equations 2:

chroma = $\sqrt{a*^2 + b*^2}$

Equation 3:

hue angle = $\tan^{-1}(\frac{a}{b})$

4.2.8 Antioxidants

4.2.8.1 Ascorbic Acid

Vitamin C was determined following the AOAC 967.21, (2000) standard method. The indicator used was 2,6-dichoro-indophenol in a titration method. Two grams of flower were extracted with 5 ml metaphosphoric acid, homogenized and filtered with Whatman paper No.4. The filtrate was taken to a volume of 25 ml in a volumetric flask with deionized water. Afterward, 5 ml of metaphosphoric-acetic acid and 2 ml of flower extract was titrated with a previously standardized dye using ascorbic acid (Figure 11). Each sample was titrated with dye until a light rose-pink color persisted. Calculation of Vitamin C in sample was carried out according to standard method established by 2,6-dichoro-indophenol method (Equation 4). This was expressed as mg ascorbic acid/100 g FW.

Equation 4:

mg ascorbic acid/ml = $[(X - B) * (\frac{F}{E}) * (\frac{V}{Y})]$

Where,

X = average ml for test solution titration

B = average ml for test blank titration

F = mg ascorbic acid equivalents to 1.0 ml indophenol standard solution

E = sample weight (g) or volume (ml)

V = volume of initial test solution

Y = volume of test solution titrated

Figure 11: Vitamin C samples

4.2.8.2. Beta-carotene

Total beta-carotene was determined following the method established by Nagata & Yamashita (1992). One gram of flowers was homogenized in 20 ml of acetone-hexane (4:6) solution and extraction was carried out using a stand dispersion unit (Polytron PT 2500 E, Kinematica, AG) for 2 minutes at 18,000 rpm (

Figure ***12***). The extract was read at 663, 645, 505, and 453 nm using a UV-spectrophotometer (UV-3100PC Spectrophotometer, Radnor, PA). Total β-carotene was calculated following the equation 5 and expressed as mg/100 g FW:

Equation 5:

$$\text{Beta-carotene (mg/100ml)} = 0.216_{(A663)} - 1.22_{(A645)} - 0.304_{(A505)} + 0.452_{(A453)}$$

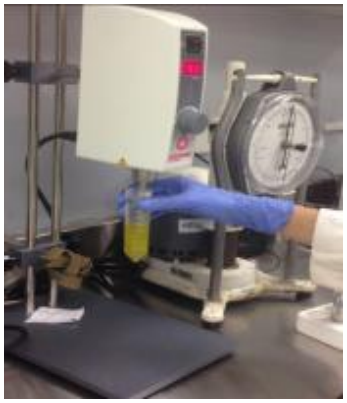


Figure 12: Homogenization of flower sample with Polytron

4.2.8.3. Total Phenolic Content

For the polyphenolic extraction, the male flowers from each genotype, that were harvested or stored, were blended. A 0.250g of sample was placed in centrifuge tubes and mixed with 2.5 ml of 80% ethanol. The ethanol extract was stirred for 24 hrs and centrifuged (IEC-HEN SII Centrifuge, Damon/IE Division, Massachusetts) at 3,000 rpm for 20 min at 25°C. The ethanol extract was then transferred to amber vials (Aquino-Bolaños et al., 2013).

Total phenolic content was determined by the Folin-Ciocalteau (F-C) colorimetric method. A 20 µl of sample was placed into a 2 ml glass cuvette with 1.58 ml of water, followed by 100 µl of F-C reagent. The sample was mixed thoroughly by inverting and incubated for 1 to 8 min. Then, 300 µl of sodium carbonate was added, and the solution was again mixed and incubated 2 hrs. at room temperature. Samples were measured at an absorbance of 765 nm. All measurements were done in triplicate and expressed as mg GAE /100g FW.

4.2.8.4. Antioxidant Activity (1,1-Diphenyl-2-picryl-hydrazyl (DPPH) Method)

Antioxidant activity was determined following the procedure established by Brand-Williams, Cuvelier, & Berset (1995). A 100 µl of ethanol extract was mixed with 3,900 µl DPPH reagent (3.9 mg/100 ml methanol), placed in a 4 ml plastic cuvette and incubated (in the dark) at room temperature for 30 min to measure decrease in absorbance. Absorbance was read at 517 nm

and values were expressed as Trolox equivalents (μM TE/100 g FW) using a Trolox calibration curve through linear regression (Equation 6) (Padmanabhan & Jangle, 2012):

Equation 6:

$$\% \text{ Inhibition} = \frac{(AB - AA)}{AB} * 100$$

Where,

A_B = absorption of blank sample (t = 0 min)

A_A = absorption of test extract solution (t = 30 mins)

4.3. Mineral Analysis

Minerals such as calcium, iron, magnesium, phosphorous, potassium, sodium and selenium were evaluated by Inductively Coupled Plasma (ICP). Previously digested ash samples of pumpkin flowers were classified for minerals following the method established by the company Perkin-Elmer, (1994). Samples of 1.000 g of pumpkin flowers of each genotype previously washed with demineralized water were incinerated at 500˚C for 4 hrs and let to cool at room temperature. Then, the digestion process was carried out using 20 ml of HCl 33% on a hot plate until half of the sample's content was reduced (about 10 ml). After digestion, the samples were filtered using Whatman paper No.541 in 100 ml volumetric flasks. Washes with demineralized water were carried out until completing volume in the volumetric flask. Content of minerals were evaluated by using an ICP Optimal Emission Spectrometer (Perkin Elmer Optimal 7300 DV, MA). Concentrations (ppm) were determined using a standard curve with the elements wanting to be quantified.

4.4. Proximal Analysis

Moisture content was determined by the (AOAC 1990, 1990) method AOAC 925.04, ash by AOAC 923.03, protein by AOAC 991.20, crude fiber by AOAC 992.16, and fat by Ankom method (AOAC 920.39).

4.5. Canning Process

The stamen was removed from all flowers. Flowers were cleansed of any unwanted material, disinfected with chlorine 200 ppm in one liter of water for 1 min, re-washed with water, and blanched for 1 min and 50 s at 100°C. To obtain the optimum time of blanching, flowers were boiled at 100°C for 1 min, 1.25 min, 1 min and 50 s, and 2 min. At 1 min and 50 s flowers still had a good appearance and texture. This is the time that was used for further evaluations. Immediately, flowers where thermally shocked for 2 min to avoid excessive softening of tissue. Then, flowers were drained to remove excess water and placed into a glass jar with a hot 1.8% saline solution (7 g of salt added into 330 g of distilled water) leaving a headspace. The FAO, (1995) recommends that the saline solution be between 2 to 3% and be heated until a temperature of 82°C is reached. A 2 to 3% of saline solution gives a strong salt taste, therefore a saline solution of 1.8% was used with the blanched pumpkin flowers. The pH of the solution was measured and adjusted to 4.6 with 1% citric acid. Initial pH was 6.53, and with the addition of citric acid 1%, dropped to 4.47. Finally, the glass jar was sealed. For the sealing process, the jars were placed in a water bath at 82°C for 5 minutes and let to cool at room temperature (Codex Alimentarius, 2011). The canned flowers were stored at room temperature for further sensory evaluation.

4.5.1. Peroxidase Inactivation

To test whether blanching was successful, enzymatic activity was measured by the AOAC 963.27 standard method. A 5-ml distilled water was placed in a test tube with 1 ml catechol, 1 ml hydrogen peroxide, and a piece of blanched pumpkin flower for 5 min. Flower tissue presenting many reddish-brown spots was considered positive for enzymatic activity (

Figure ***13***).

Figure 13: Peroxidase inactivation of male pumpkin flowers.

4.6. Sensory Analysis

Preliminary tests with an untrained group of 5 to 10 people were carried out to determine which genotypes to use in the sensory evaluations panels. Based on these tests, the *C. pepo* genotypes (Bush White Scallop and Early Prolific Straight-neck) were not included in the sensory evaluation, while Taina Dorada, Verde Luz and Soler were included. Due to the limited availability of flowers, the sensory tests were carried out with a mixture of flowers from those genotypes. A minimum of 100 subjects were used for each sensory panel, and the participants were of both sexes, of various ages, and included both UPRM students and employees. Participants were pre-screened, and only those who indicated that they liked vegetables like lettuce were asked to participate. All sensory analysis took place at the UPRM campus. Texture, taste and general acceptance was evaluated on fresh flowers but for cooked (canned) flowers only general acceptance was evaluated. In both cases, a 9-point hedonic scale where 9 = like extremely; 7 = like moderately; 5 = neither like nor dislike; 3 = dislike moderately; and 1 = dislike extremely (Meilgaard & Muller, 1987) was used. Panelists were given one to two petals when evaluating freshly harvested pumpkin flowers and one cooked flower when evaluating minimally processed flowers. The sensory evaluation form appears in Appendix 8.5. Overall acceptability of cooked flowers was evaluated in the lobby of the Piñero building on 24 April 2015 using 127 panelists. Texture of fresh flowers was evaluated in the lobby of the Piñero building on 28 April 2016 using

103 panelists. Taste of fresh flowers was evaluated in the lobby of the Chemistry building on 2 May 2016. Lastly, overall acceptability evaluated in the lobby of Piñero and in the Food Science and Technology building on 9 May 2016 with a total of 118 panelists.

4.7. Data Analysis

Data was analyzed as a complete block design with three replications (three extracts, samples and results) and a factorial arrangement of treatments (four genotypes by three storage treatments) for all physicochemical measurements. All statistical analysis was carried out using InfoStat (version 2015e). F tests were used to test the significance of interaction and main effects and means were separated using Tukey's least significant difference comparison test. A simple ANOVA was used to determine differences between genotypes when evaluating yield performance (average weight and length) of flowers. The correlation between pH and total acidity was done using the Pearson correlation coefficient (r).

5. Results and Discussion

5.1. Yield of different genotypes of pumpkin flowers

During the months of May to June 2015, and starting at 29 days after transplanting, the five tested genotypes (Table 9 in Appendix) were harvested on a total of 27 different days (
Figure ***14***). Bush White Scallop and Early Prolific Straight-neck began to produce flowers (on day 29 and continued producing more flowers than the other genotypes (until 65 days after transplanting when their production of flowers decreased drastically). Taina Dorada, Verde Luz, and E1101-1 began to flower 50 days after transplanting. Verde Luz was the last genotype to flower (at day 65). Bush White Scallop produced an average of 16.11 flowers (Table 4) per day and average of 4 flowers per plant per day. This genotype produced more flowers than the other genotypes. Yield of female flowers was also evaluated. Bush White Scallop and Early Prolific Straight-neck were the first to flower beginning 36 days after transplanting, and produced more female flowers than the other genotypes. After day 60, their production of female flowers began to decrease. On day 43 after transplanting, E1101-1 began to flower. At day 44, Verde Luz and Taina Dorada, flowered. There was a production of up to 4 flowers of female flowers per plant during the 27 days of harvesting. Early Prolific Straight-neck produced an average of 1.11 flowers per plant per day, significantly different from other genotypes. Verde Luz produced the least number of female flowers per plant per day.

A study carried out by Kiramana et al. (2016), evaluated the yield performance of male and female pumpkin flowers (*C. moschata*) in 72 and 79 accessions planted in two different farms located in Kenya. The total number of male flowers per plant ranged from 11 to 197 during the two seasons of the harvesting period (harvesting began 20 days after planting). Total female flowers per plant ranged from 1 to 10 over the total harvest period.

Table 4: Mean number of male flowers produced per day per plant, and individual flower weight and length in five *Cucurbita* genotypes harvested on 27 different days in May and June 2015 in Lajas, Puerto Rico.

Genotype	*Cucurbita* Species	Average number of flowers per plant		Average number of flowers per day		Average flower weight (g)		Average flower length (cm)	
Bush White Scallop	*C. pepo*	16.11	a	4.03	a	2.93	c	8.54	c
Early Prolific Straightneck	*C. pepo*	7.13	c	1.78	c	3.29	c	8.47	c
Taína Dorada	*C. moschata*	13.51	ab	3.38	ab	8.05	ab	11.33	b
Verde Luz	*C. moschata*	13.68	ab	3.42	ab	8.80	ab	12.47	a
E1101	*C. moschata*	11.77	b	2.94	b	7.87	b	12.71	a
LSD Tukey (0.05)		4.050		1.013		0.771		0.561	
F test (probability)		<0.0001		<0.0001		<0.0001		<0.0001	

Tukey-LSD: Least significant difference between means using the Tukey test at the 5% probability level. Within a column, means followed by a common letter are not different at the 5% probability level.

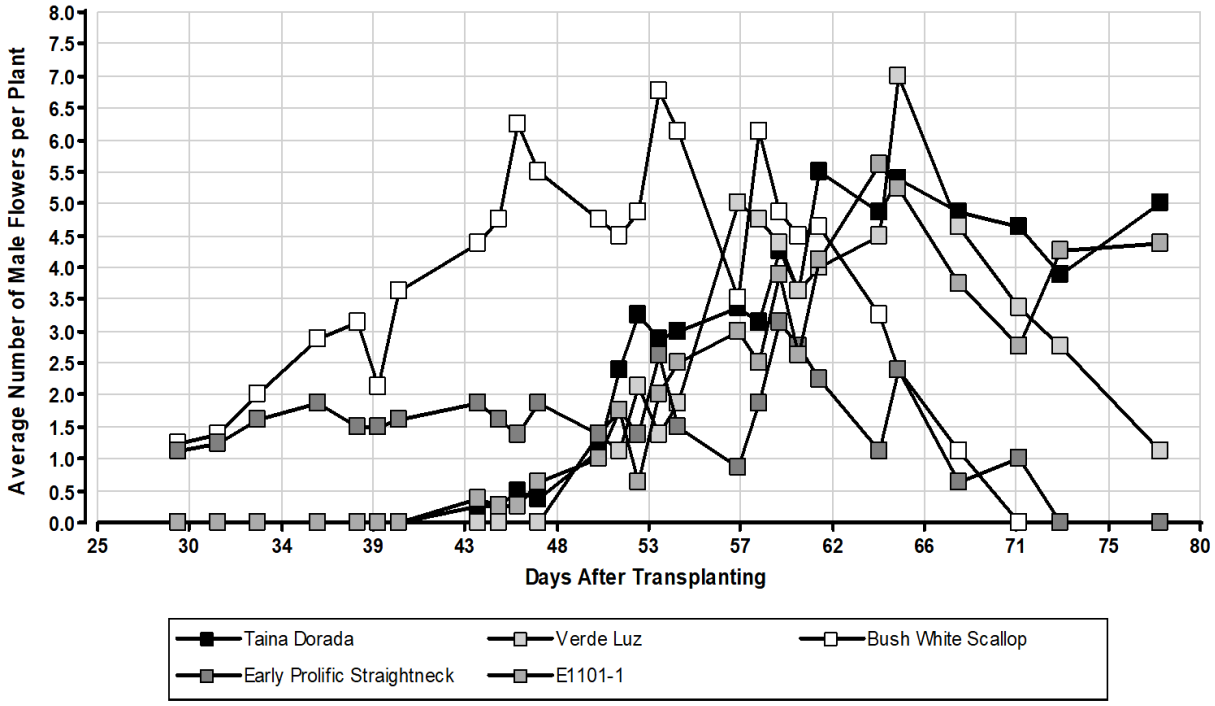


Figure 14: Average number of male flowers per plant in five *Cucurbita* genotypes evaluated from 29 to 80 days after transplanting during May and June 2015 in Lajas, Puerto Rico

Over the 27 days of harvesting, Taina Dorada, and Verde Luz, produced flowers with the most weight. These had an average weight of 8.05 g and 8.80 g, respectively per day. Bush White Scallop and Early Prolific Straight-neck had an average flower weight of 2.93 and 3.29 g, respectively per day. It can be noted that these weights are maintained during the 27 days of harvesting (

Figure ***15***). Flower weight and length of Verde Luz (8.80 g and 12.5 cm, respectively) was greater than that of all the other genotypes during the whole harvesting period (Table 4) but not significantly different from E1101-1. Early Prolific Straight-neck was the genotype with the shortest flowers (8.5 cm) during the 27-day harvesting period (

Figure ***16***). Other studies have shown that male flowers vary in size. Martinez Mirafuentes & Garcia Martinez, (1998 cited in Lopez,2007) observed *Cucurbita* flower lengths of 6 to 25 cm (from the tip of the petals until the peduncle). No studies on the average weight of pumpkin flowers were found.

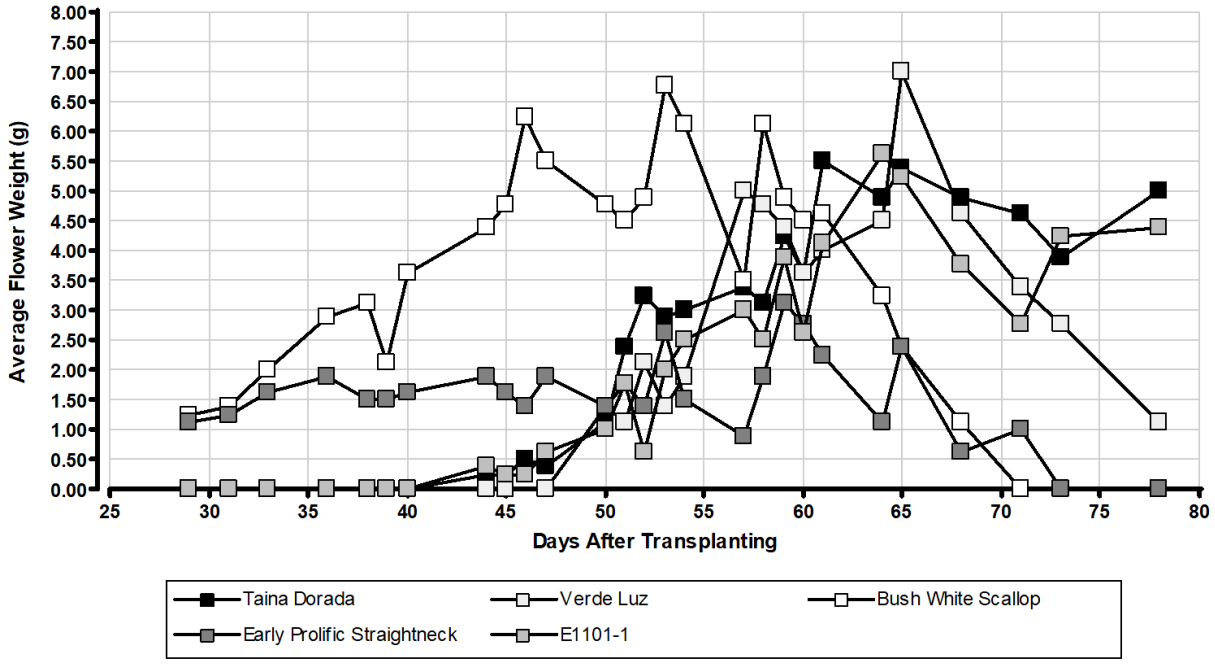


Figure 15: Average male flower weight (g) in five *Cucurbita* genotypes evaluated from 29 to 80 days after transplanting in May and June 2015 in Lajas, Puerto Rico.

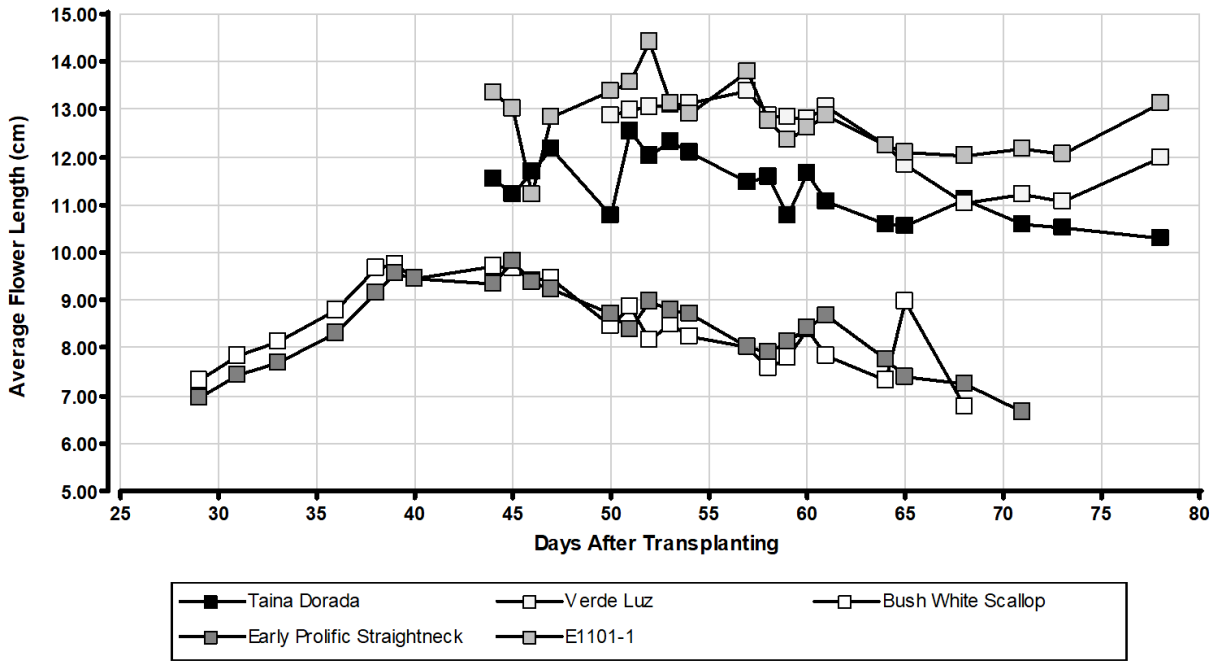


Figure 16: Average male flower length (cm) in five *Cucurbita* genotypes evaluated from 29 to 80 days after transplanting in May and June 2015 in Lajas, Puerto Rico.

5.2. Shelf life

5.2.1. Weight loss

Flowers had a non-marketable appearance starting at the 4th to 5th day of storage. For this reason, measurements were made at the 3rd day of storage. For flowers stored at both 5°C and 10°C no genotype by storage treatment interaction was observed (Table 11 and Table 12 in Appendix), and flowers in packaging with and without MAP exhibited the same weight loss. At 5°C, weight loss among genotypes ranged from 7.41 to 14.79% (Table 5). At 5°C there were no differences in weight loss among flowers of the three genotypes. At 10°C flowers of Bush White Scallop had a greater weight loss than Verde Luz but not different from Taína Dorada. Weight loss of flowers after 5 days of storage are reported even though no F test was carried out. At 5°C, weight loss ranged from 20.69 to 31.29%. At 10°C, weight loss ranged 19.57 to 37.25%. At both storage

treatments and temperatures, Bush White Scallop was the genotype to lose the most amount of weight but this was not statistically proven.

Table 5: Mean percentage weight loss of male *Cucurbita* flowers of three genotypes at 3 and 5 days post-harvest. Flowers packaged in plastic trays with and without (control) modified atmosphere packaging (MAP) at 5°C and 10°C.

	Weight loss (%)							
Effect	5°C				10°C			
	Day 3		Day 5		Day 3		Day 5	
Genotype (G)								
Bush White Scallop	14.79	a	31.29	*	17.73	a	37.25	*
Taina Dorada	9.82	b	20.69	*	14.96	ab	23.77	*
Verde Luz	7.41	b	29.29	*	7.74	b	19.57	*
F Test (p value)	0.054		*		<0.0001		*	
Tukey LSD (0.05)	na		*		8.944		*	
Treatment (T)								
Control	10.80	a	26.38	*	14.65	a	28.69	*
MAP	10.55	a	25.79	*	12.31	a	24.22	*
F test (p value)	0.426		*		0.757		*	
Tukey LSD (0.05)	na		*		na		*	
G x T								
F test (p value)	0.919				0.615			

Tukey-LSD: Least significant difference between means using the Tukey test at the 5% probability level.
na = Tukey test not applicable because F test was not significant.
Within a column, means followed by a common letter are not different at the 5% probability level.
* No F test was carried out. The numbers are only being reported.

A study evaluated by Villalta, Ergun, Berry, Shaw and Sargent (2004) demonstrated that weight loss of summer squash flowers (*C. pepo*) after 7 days of storage at 5°C without MAP was less than 3% of fresh weight. At 14 days, a total of 7.32% of fresh weight was lost, compared with 5.12% of fresh weight loss in flowers stored at 2.5°C. Aquino-Bolaños et al. (2013) demonstrated that pumpkin flowers stored with MAP at 5°C, lost 2.35% of their initial weight, while flowers stored under air lost 7.67% by day 8 of storage. In this study, Verde Luz had a weight loss of approximately 7-8% in both storage treatments at 5°C, on the 3rd day of storage. In the other

genotypes, values obtained from this study exceeded those from previous studies. Weight loss of pumpkin flowers reached up to 14.79% (at 5°C) and 17.73% (at 10°C) on the 3rd day of storage. López-Puc and Rodríguez-Buenfil (2015) evaluated the weight loss of cut gladious (*Gladiolus grandiflorus*) ornamental flowers stored with different mixtures of MAP at 0 and 5°C. In that study, the weight loss of flowers was less at 5°C with a MAP mixture of 90% N_2 and 10% CO_2. At 0°C, weight loss was less when stored with a MAP mixture of 70% N_2, 15% CO_2 and 15% O_2. Weight loss of these flowers can be attributed to the fact that low temperature is needed to retain quality, since by retaining weight, heat damage is reduced. Low temperature has an impact on slowing several metabolically processes such as, delaying floral aging (Lopez-Puc & Rodriguez-Buenfil, 2015).

5.2.2. Visual Quality

Flower were considered marketable when rated 4 (petals curled slightly) or 5 (no defects). Within the genotypes the pattern of quality loss was similar in both storage treatments and at both temperatures (5°C and 10°C), but there were differences among genotypes (

Figure ***17***,

Figure ***18***,

Figure ***19*** and

Figure ***20***). From the 4th to 5th day of storage, the visual quality rating of Taina Dorada, Verde Luz and Soler averaged 3.17 (petals curled moderately). In contrast, after 5 days of storage Bush White Scallop, had an average rating of 1 (petals wilted and decayed) for samples without MAP and 2.5 (ranging from petals curled moderately to petals curled severely) for samples with MAP. Flowers with a rating of 2.5 could be considered marketable depending on the client's perspective. After the 6th day of storage, visual quality of flowers of all genotypes began to decrease drastically making them unmarketable. On the 7th day of storage, flowers were rated as 1, meaning they were completely wilted and signs of decay had begun. There was no significant difference found for flowers stored without and with MAP at both temperatures. Comparing flowers without and with MAP, samples with MAP had a slightly better visual quality than those without MAP at both temperatures. At 5°C, the visual aspect of flowers was better than at 10°C

for both storage treatments. Pumpkin flowers in this study couldn't be stored for more than 5 days in contrast with studies done by Villalta et al. (2004) and Aquino-Bolaños et al. (2013).

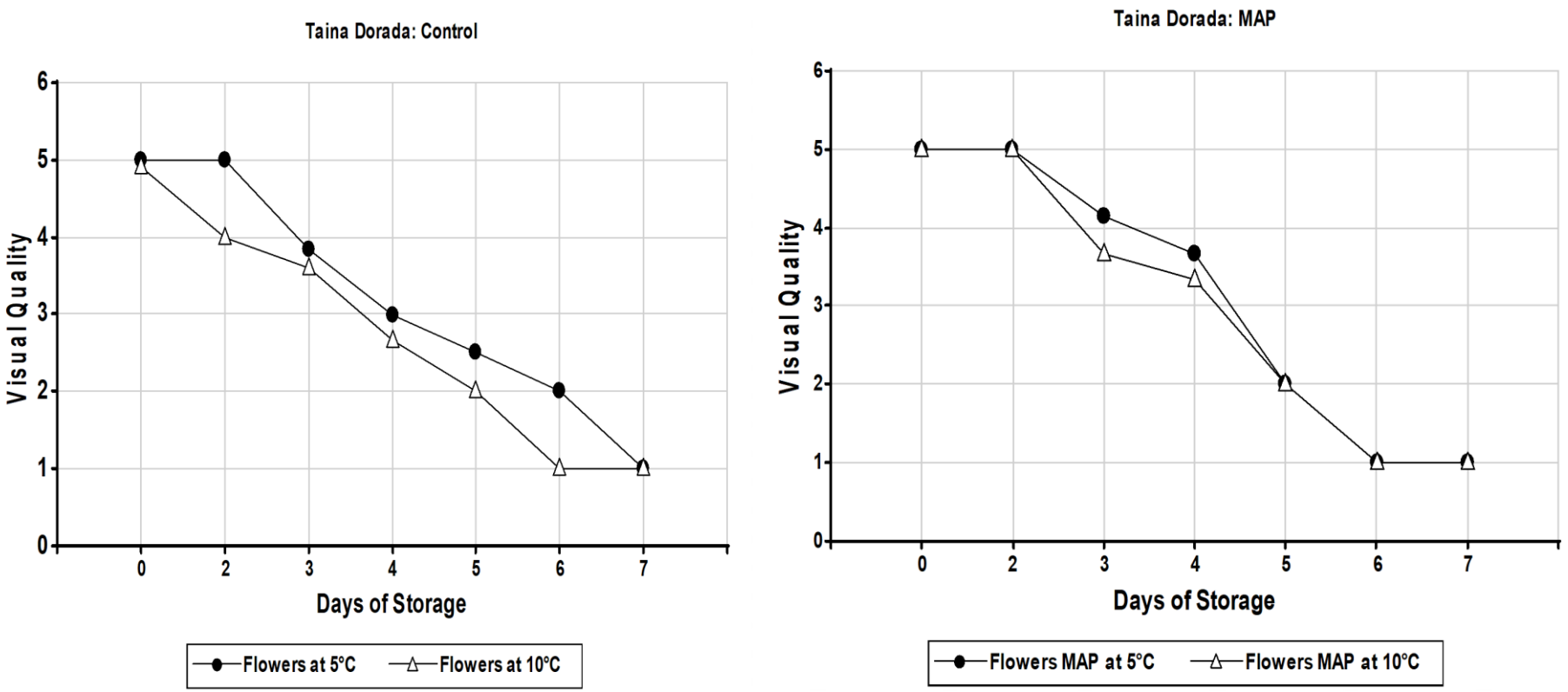


Figure 17: Mean visual quality over 7 days for male flowers of Taína Dorada stored (a) without modified atmosphere packaging (MAP) and (b) with MAP. Visual quality scale was from 1 (flowers wilted and decayed) to 5 (no defects).

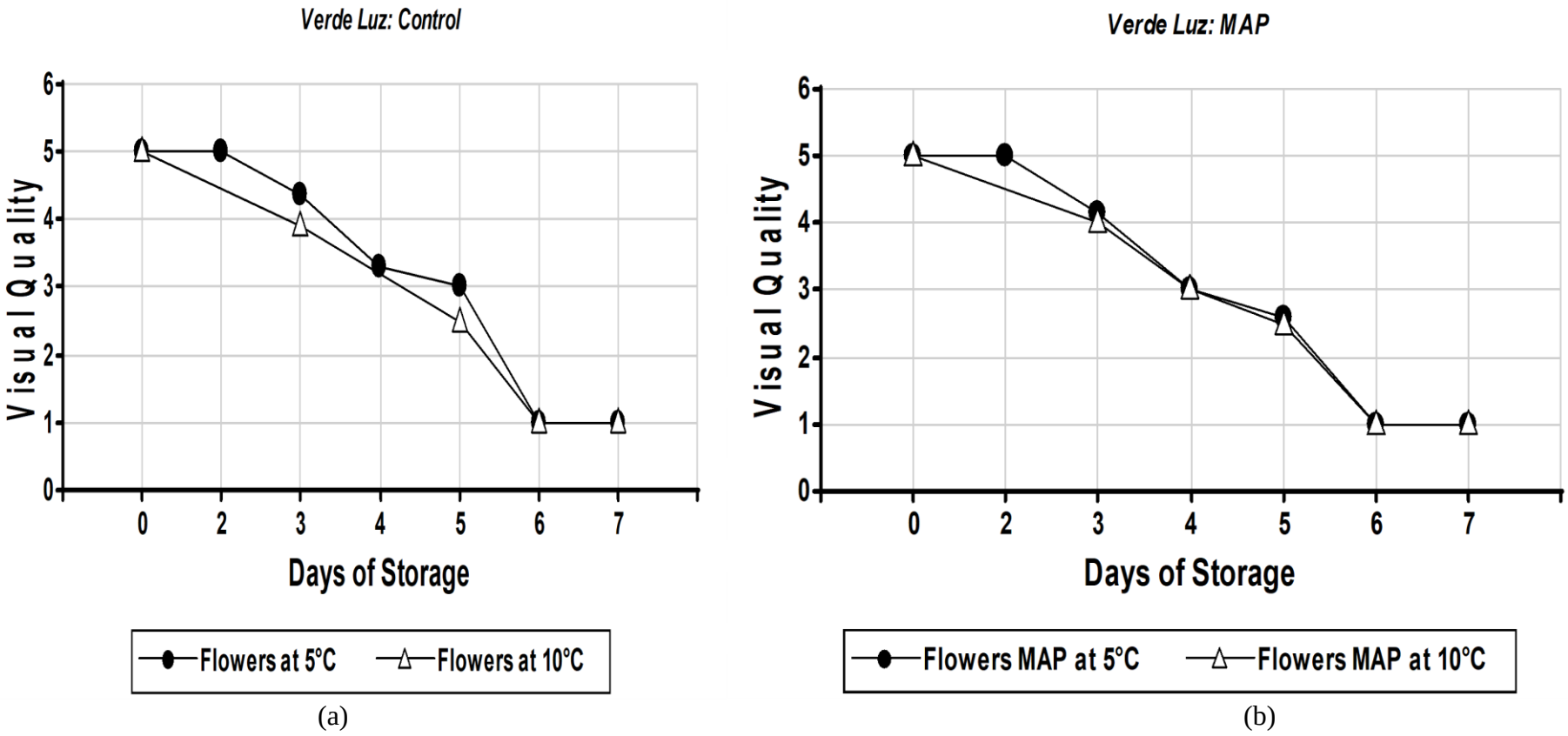
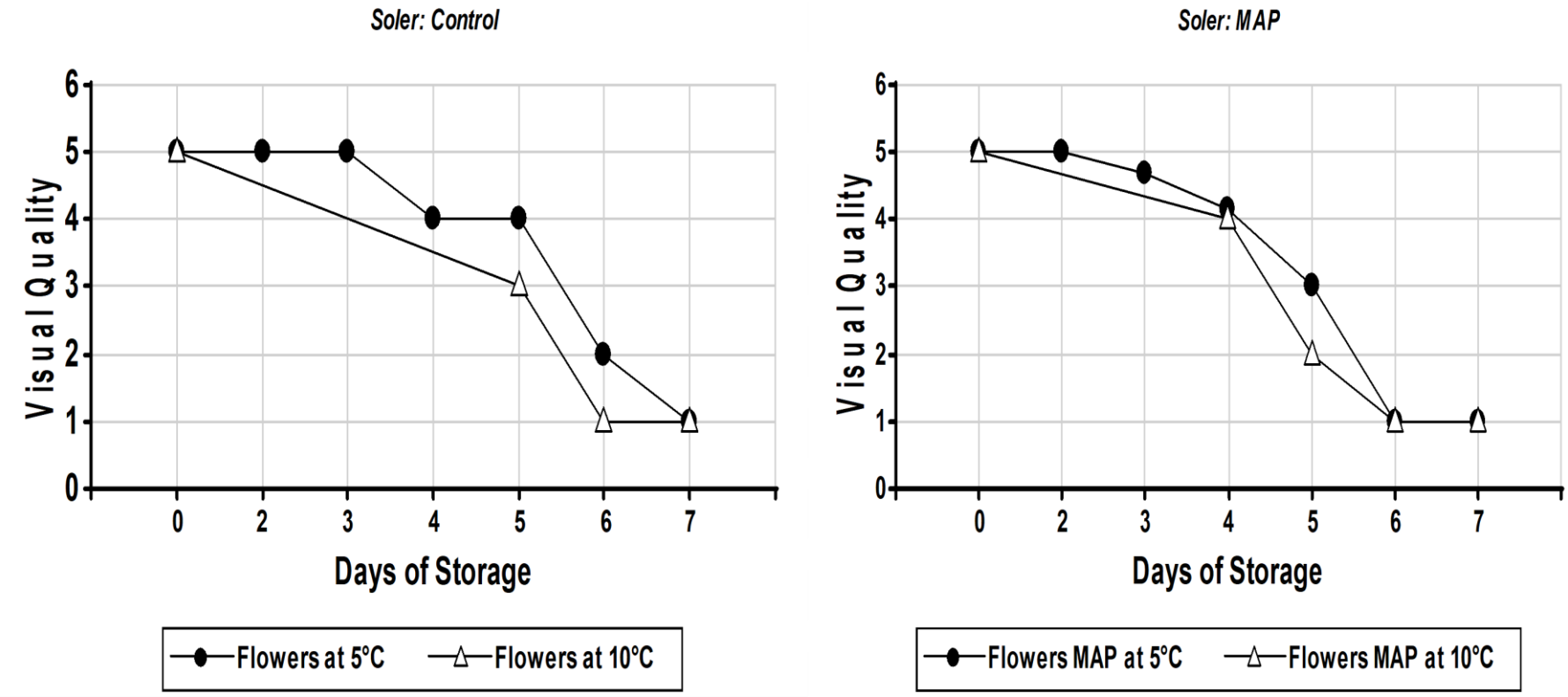


Figure 18: Mean visual quality over 7 days for male flowers of Verde Luz stored (a) without modified atmosphere packaging (MAP) and (b) with MAP. Visual quality scale was from 1 (flowers wilted and decayed) to 5 (no defects).



(a) (b)

Figure 19: Mean visual quality over 7 days for male flowers of Soler stored (a) without modified atmosphere packaging (MAP) and (b) with MAP. Visual quality scale was from 1 (flowers wilted and decayed) to 5 (no defects).

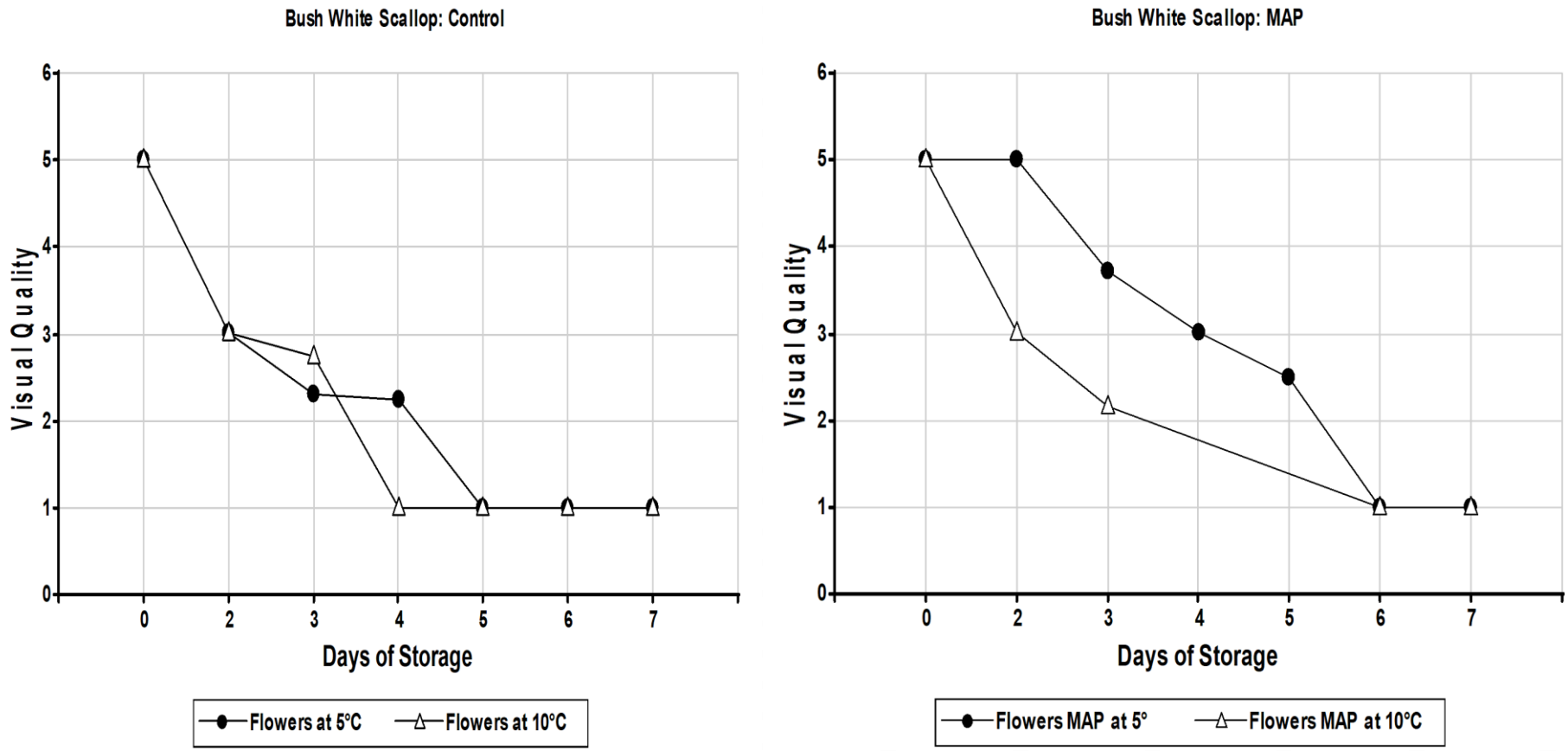


Figure 20: Mean visual quality over 7 days for male flowers of Bush White Scallop stored (a) without modified atmosphere packaging (MAP) and (b) with MAP. Visual quality scale was from 1 (flowers wilted and decayed) to 5 (no defects).

5.2.3. CO_2 and O_2 Concentration Variations during Storage

For CO_2 and O_2, the relative differences varied depending on genotypes and storage treatment (the genotype by storage treatment interaction (Table 11 and Table 12 in Appendix) was significant). Samples stored under MAP had a mixture of 5% O_2, 11% CO_2, and 84% N_2 while samples without MAP only had air. Initially, samples without and with MAP at both storage temperatures had no significant differences between genotypes. At time zero, the average concentration of CO_2 at 5°C was 0.96% (without MAP) and 11.50% (with MAP). The average concentration of CO_2 at 10°C was 0.77% (without MAP) and 11.46% (with MAP). After 5 days of storage at 5 and 10°C, there was an increase of CO_2 for both storage treatments and for all genotypes. Containers with Soler flowers had a higher CO_2 concentration than all the other genotypes. The O_2 concentration decreased after storage for both packaging treatments and temperatures. Soler was the genotype that lost most O_2 concentration.

In both storage treatments, and at both temperatures, there was an accelerated decrease in O_2 and increase in CO_2 after the second day of storage. At 5°C and 10°C, for O_2 concentration, there was a decrease ranging from approximately 6% to 2-4% for MAP samples. In contrast, samples without MAP, decreased from 21 to 13-15% in both temperatures after the second day of storage. After 7 days, O_2 was almost completely consumed in both storage treatments and temperatures. Samples without and with MAP, consumed slightly less O_2 at 5°C than at 10°C.

CO_2 concentration, was higher for both storage treatments at 10°C. This can be attributed to the fact that increase temperatures result in higher levels of respiration (Teixeira, 2016). In this process stored organic materials (carbohydrates, proteins, fats) are broken down into simple end products with a release of energy. Oxygen (O_2) is used and CO_2 is produced during this process (Kader, 1989). There was a CO_2 increase in both storage treatments from 0.9 up to 13% for samples without MAP on the second day of storage for both temperatures. For MAP samples, there was an increase from 11% up to 19% for both temperatures. After 7 days of storage, for both storage treatments and temperatures, there was a production of CO_2 of approximately 22%. There were no significant differences in storage temperature for samples stored without and with MAP and how this affected their CO_2 and O_2 concentration.

The results are similar to those of previous studies where O_2 decrease and CO_2 increase after storage for all genotypes: Taína Dorada (

Figure **21**), Verde Luz (

Figure **22**), Soler (

Figure **23**), and Bush White Scallop (

Figure **24**). Kou and contributors (2012), studied the shelf life of edible carnations and snapdragon flowers applying MAP and 1-methylcicloprοpene. These edible flowers had a decrease in O_2 concentration from the 1st day of storage until the 7th and 14th day. From day 7 to 14, CO_2 accumulated in snapdragons while in carnations it decreased due to a reduction in respiration rate. Previous studies also demonstrated that storage of dry-sale Asiatic hybrid lily bulbs (*Lillium marseille, L. vivaldi, and L. vermeer*) in a 1% O_2 atmosphere improved the shelf life and quality in comparison of storage with air. This research stated that the success of a MAP system can be dependent on the tolerance of lily buds to elevated CO_2 levels that would occur in MAP (Legnani, Watkins, & Miller, 2004). A study evaluated on *Tropaeolum majus L*. edible flowers, demonstrated that storage at 2 to 5°C, and with MAP did not give a significant effect on extending the shelf life of flowers. Storage at 10% O_2 and 5% CO_2 improved the flower quality only very slightly (Friedman et al., 2005). This can explain the similar behavior observed for samples without and with MAP in this study. Storage of different types of flowers will vary depending on flower species and gas concentrations (Halvey & Mayak, 1989).

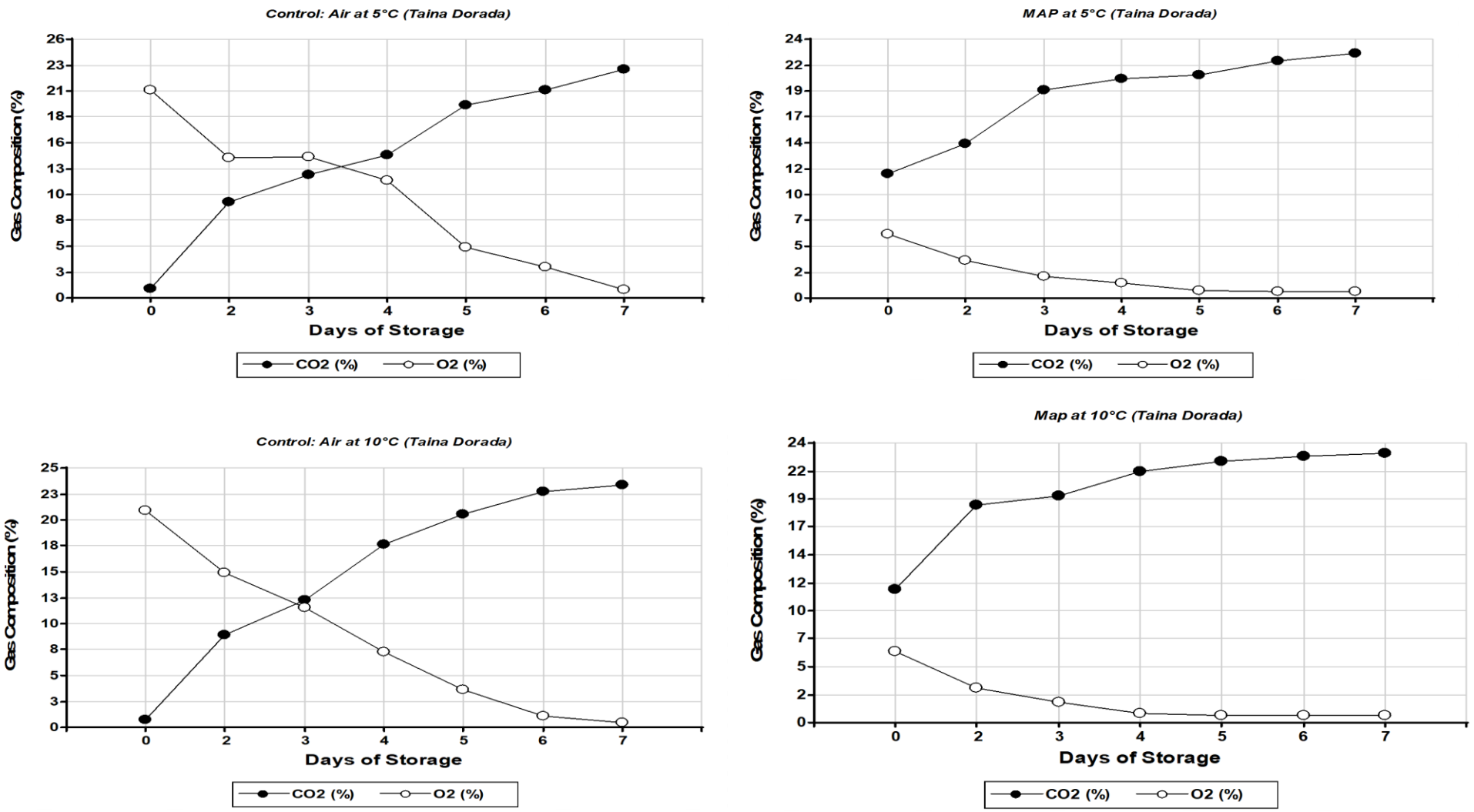


Figure 21*:* Changes in CO_2 andO_2 concentrations in packages of male flowers of Taína Dorada with and without modified atmosphere packaging (MAP) stored at 5°C or 10°C for 7 days.

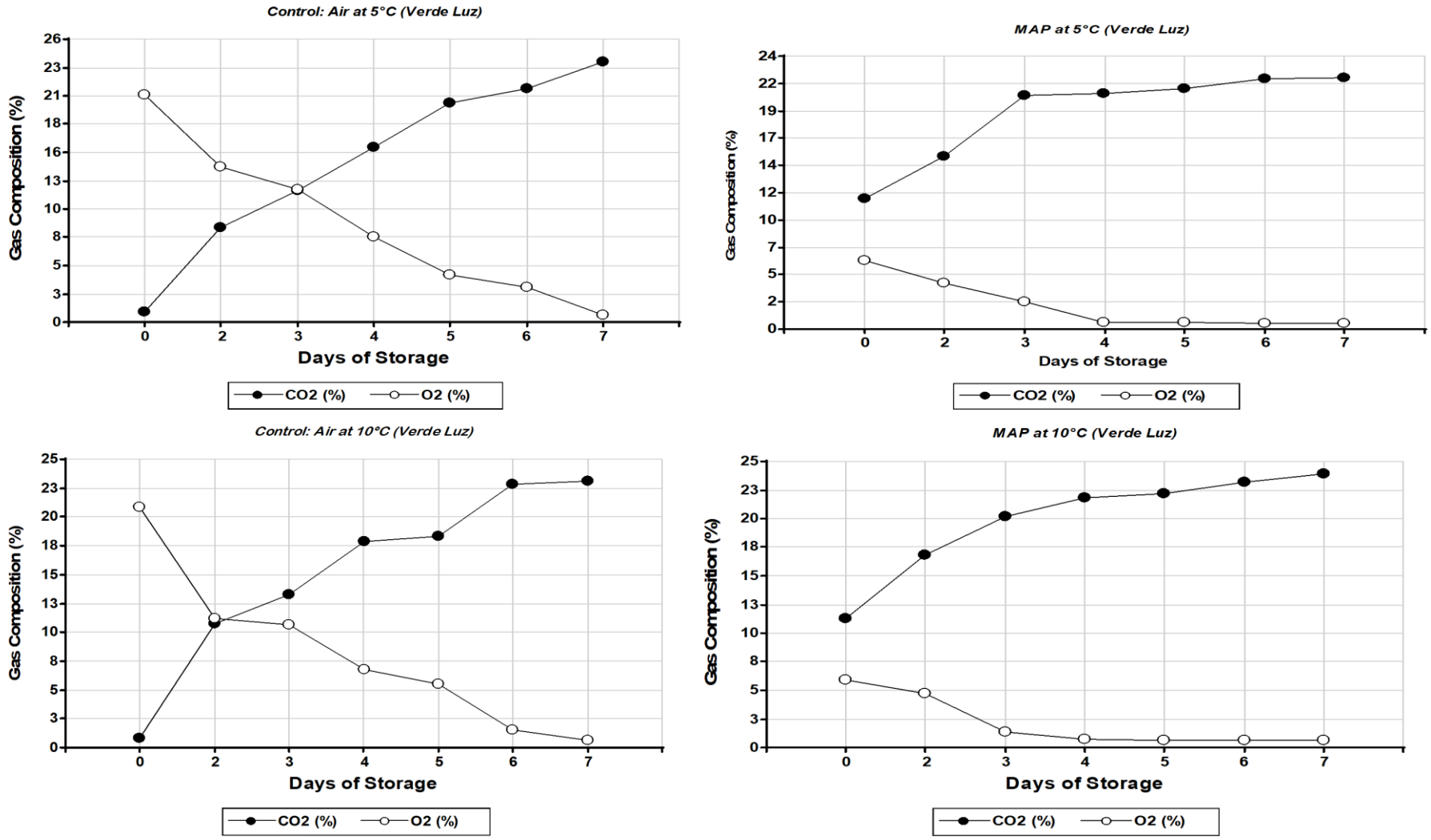


Figure 22*:* Changes in CO_2 andO_2 concentrations in packages of male flowers of Verde Luz with and without modified atmosphere packaging (MAP) stored at 5°C or 10°C for 7 days.

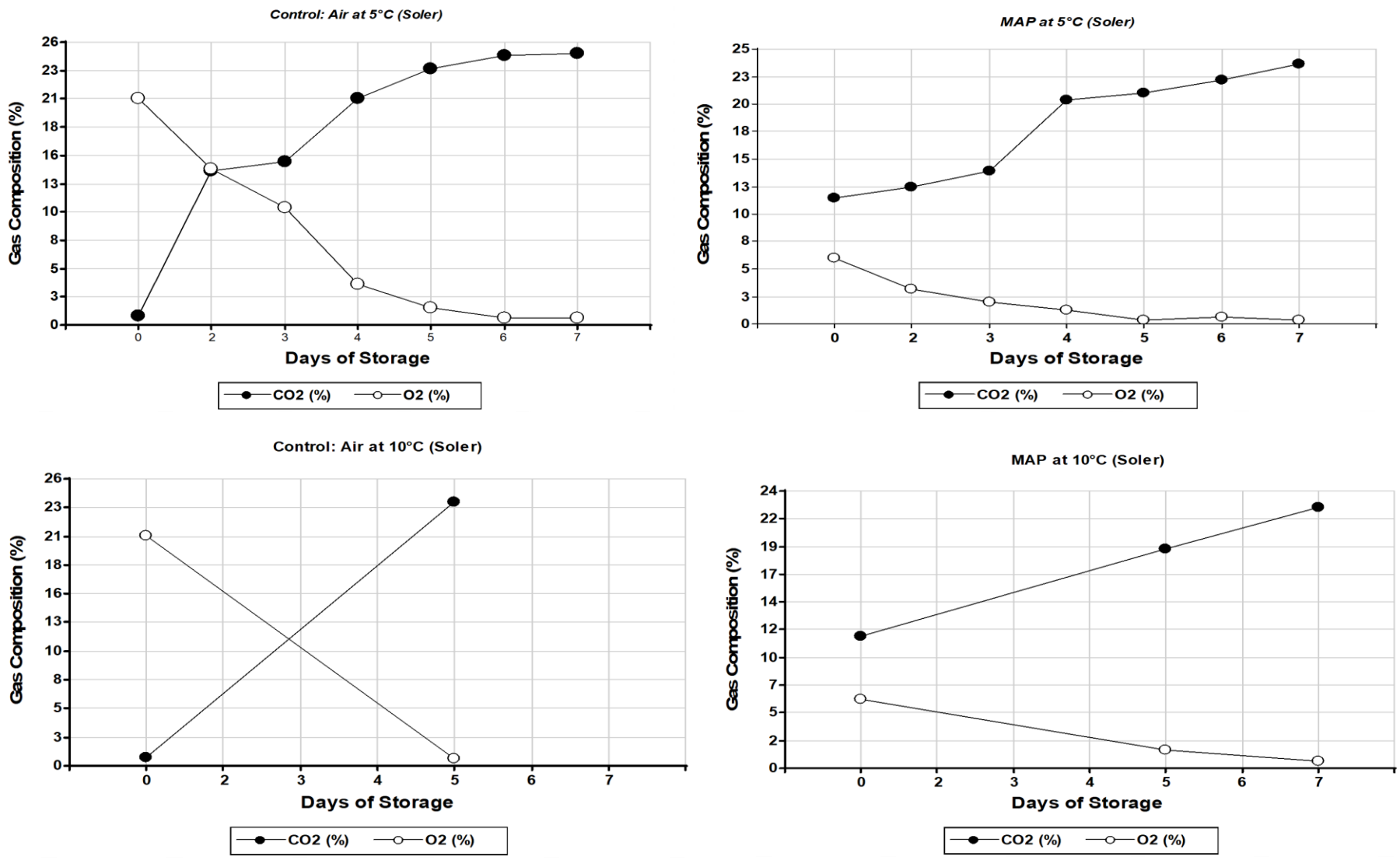


Figure 23: Changes in CO_2 andO_2 concentrations in packages of male flowers of Soler with and without modified atmosphere packaging (MAP) stored at 5°C or 10°C for 7 days.

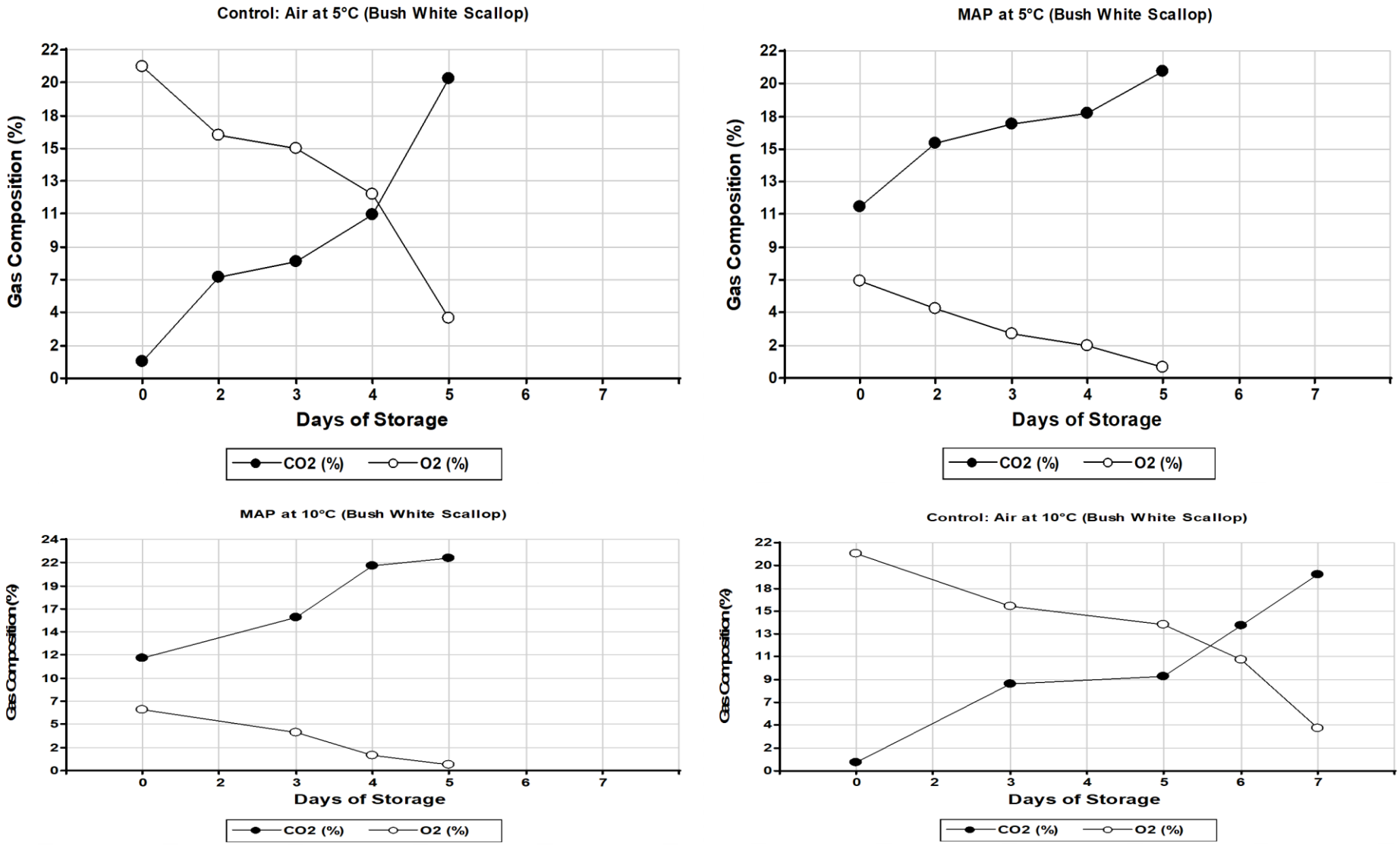


Figure 24: Changes in CO_2 and O_2 concentrations in packages of male flowers of Bush White Scallop with and without modified atmosphere packaging (MAP) stored at 5°C or 10°C for 7 days

5.2.4. Storage

Because the tested flowers did not maintain an acceptable visual quality for 14 days, the evaluation period was reduced to 7 days. The series of photographs in figures 24 to 27 document the appearance of typical flowers stored at 5°C and 10°C in either non-MAP or MAP over the 7-day period. On the 3rd to 4th day of storage at both 5°C and 10°C, flowers had begun to lose water, and petals had begun to curl slightly or moderately. On the 5th day, severe in-rolling of petals and signs of wilting had occurred on the flowers making them unmarketable. At 5 days, flowers stored at 5°C had a better visual appearance than flowers stored at 10°C. Low storage temperatures reduce respiration, thus improving the product's quality (Teixeira da Silva, 2003).

The results of this research did not agree with previous studies where good quality pumpkin flowers were maintained for a much longer period. Aquino-Bolaños et al., (2013) stored male flowers at 5°C and these had a shelf life of 14 days. Villalta et al. (2004) stored closed (pre-anthesis) male and female flowers at 2.5°C and 5°C without MAP. These flowers had a shelf life of 7 days in a marketable state. Different species of edible flowers subjected to low storage temperature will respond differently (Villalta et al., 2004). Kelley et al. (2002) demonstrated that flowers of viola, pansy and nasturtium were marketable after 2 weeks of storage at -2.5°C to 2.5°C but only one week at temperatures above 2.5°C. Scarlet runner bean blossoms, where marketable for only one week at temperatures from 0 to 10°C.

(a) Day 1 (b) Day 2 (c) Day 3 (d) Day 4

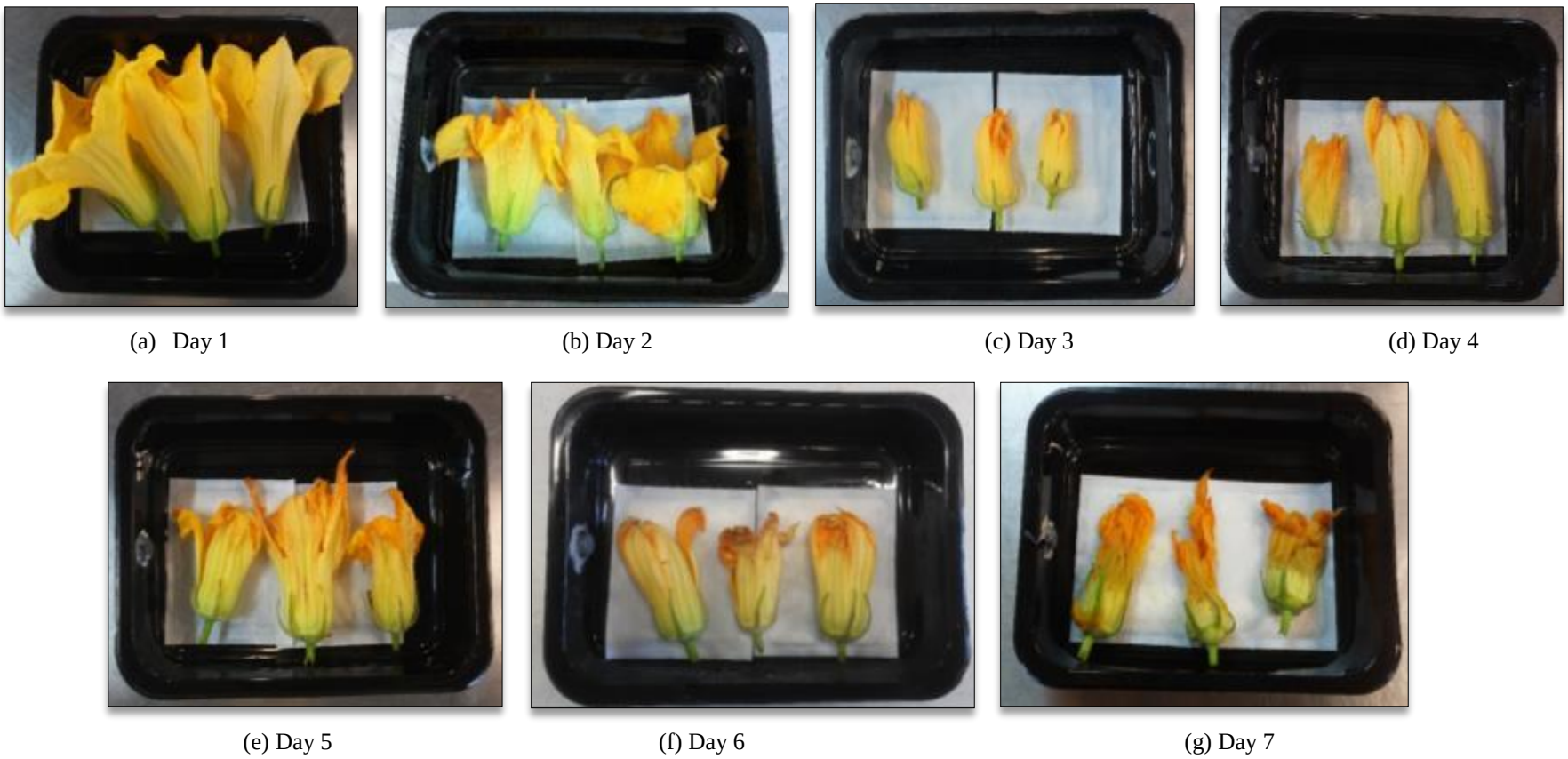
(e) Day 5 (f) Day 6 (g) Day 7

Figure 25: Male pumpkin flowers stored without modified atmosphere packaging (MAP) at 5°C for 1 to 7 days.

(a) Day 1 (b) Day 2 (c) Day 3 (d) Day 4

(e) Day 5 (f) Day 6 (g) Day 7

Figure 26: Male pumpkin flowers stored under modified atmosphere packaging (MAP) at 5°C for 1 to 7 days.



(a) Day 1 (b) Day 2 (c) Day 3 (d) Day 4

(e) Day 5 (f) Day 6 (g) Day 7

Figure 27: Male pumpkin flowers stored without modified atmosphere packaging (MAP) at 10°C for 1 to 7 days.

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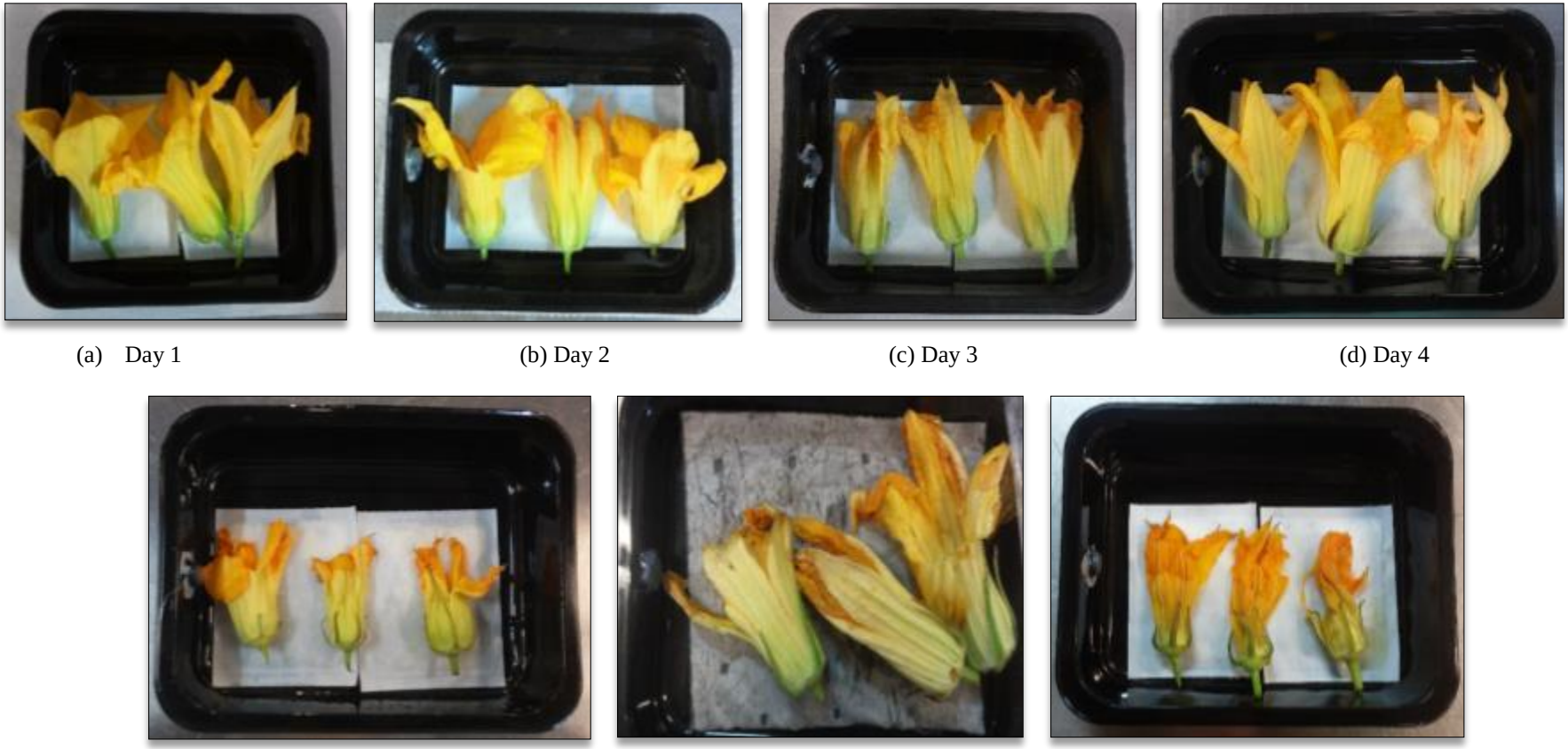

(a) Day 1 (b) Day 2 (c) Day 3 (d) Day 4

(e) Day 5 (f) Day 6 (g) Day 7

Figure 28: Male pumpkin flowers stored under modified atmosphere packaging (MAP) at 10°C for 1 to 7 days.

5.3. Physicochemical Parameters of Flowers

5.3.1. Soluble Solids Content

For °Brix, the relative differences among genotypes varied depending on storage treatment (there was a significant genotype by storage treatment interaction (Table 13 and Table 14 in Appendix). Fresh flowers had a °Brix ranging from 3.33 to 4.08. Soler had a higher °Brix than the other genotypes. After 5 days of storage, °Brix for Verde Luz was significantly lower than all other genotypes for flowers stored at 5°C (

Figure **29**) and 10°C (

Figure **30**). In general, °Brix decreased in stored flowers compared to fresh flowers.

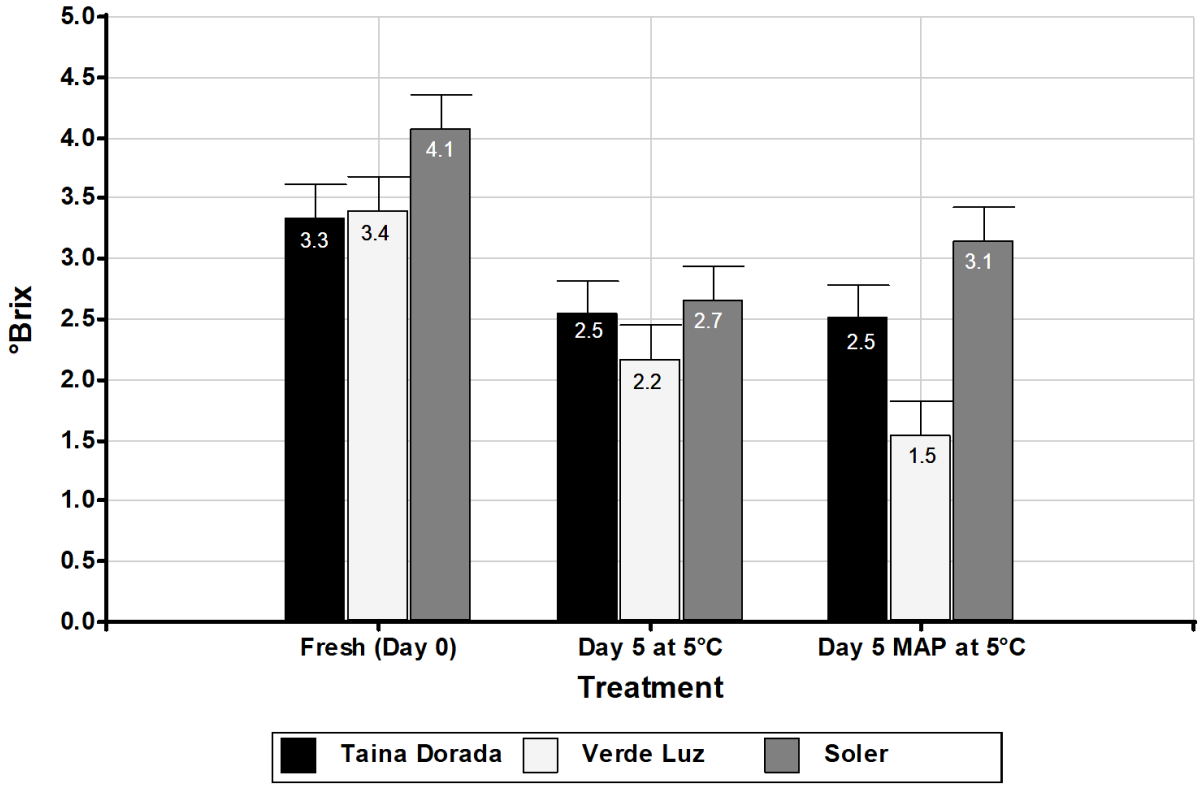

Figure 29*:* Mean °Brix in fresh flowers and flowers stored at 5°C for five days without modified atmosphere packaging (MAP) and with MAP from three genotypes of *Cucurbita moschata*. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

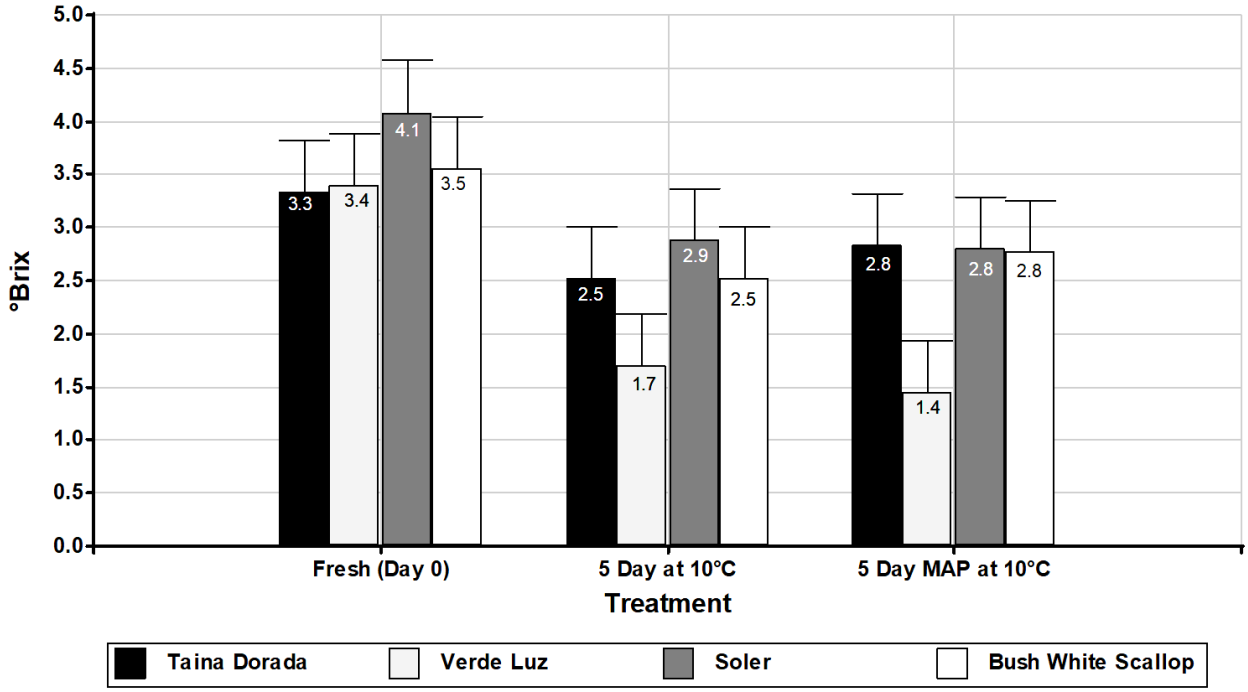

Figure 30*:* Mean °Brix in fresh flowers and flowers stored at 10°C for five days without modified atmosphere packaging (MAP) and with MAP from four genotypes of *Cucurbita moschata*. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

Initial soluble solids content was comparable to studies evaluated by Aquino-Bolaños et al. (2013) on *Cucurbita* flowers. In this same study, samples without MAP decreased from 4.57 to 1.61 °Brix after storage (8 days of storage) while MAP samples decreased from 4.57 to 3.61 °Brix after 16 days of storage. In a study of *Narcissus* cut flowers with different sour orange extract solutions to help extend the shelf life at ambient temperature, it was demonstrated that °Brix decreased over storage time (Zahra, Mehrdad, Hossein, & Atoosa, 2015).

5.3.2. Total Acidity and pH

Fresh pumpkin flowers exhibited an average total acidity content of 0.157% citric acid with no significant difference between genotypes. Total acidity content in a study of fresh chive (*Allium schoenoprasum* L.) edible flowers was 0.34% citric acid (Grzeszczuk, Weso, Jadczak, & Jakubowska, 2011). Grzeszczuk, et al.,(2016) studied various species of edible flowers and observed total acidity content ranging from 0.107 to 0.814% citric acid. Pumpkin flowers appear to be a low source of organic

acids. For samples stored at 5°C, total acidity decreased to 0.030% for samples without MAP and to 0.020% for samples with MAP (

Figure ***31***). After storage at 10°C, total acidity content of flowers decreased in all treatments to 0.020% for samples without MAP and 0.030% for samples with MAP (

Figure ***32***). Taina Dorada and Verde Luz had the least amount of total acidity in both cases.

Total acidity in this study was lower than that found in a study by Aquino-Bolaños et al. (2013) where total acidity in flowers stored without MAP decreased to 0.055% and 0.122% for samples with MAP. The decrease in total acidity can be explained by the fact that flowers with low reserve substrate tend to use the organic acids present as a substrate for respiration processes (Teixeira da Silva, 2003).

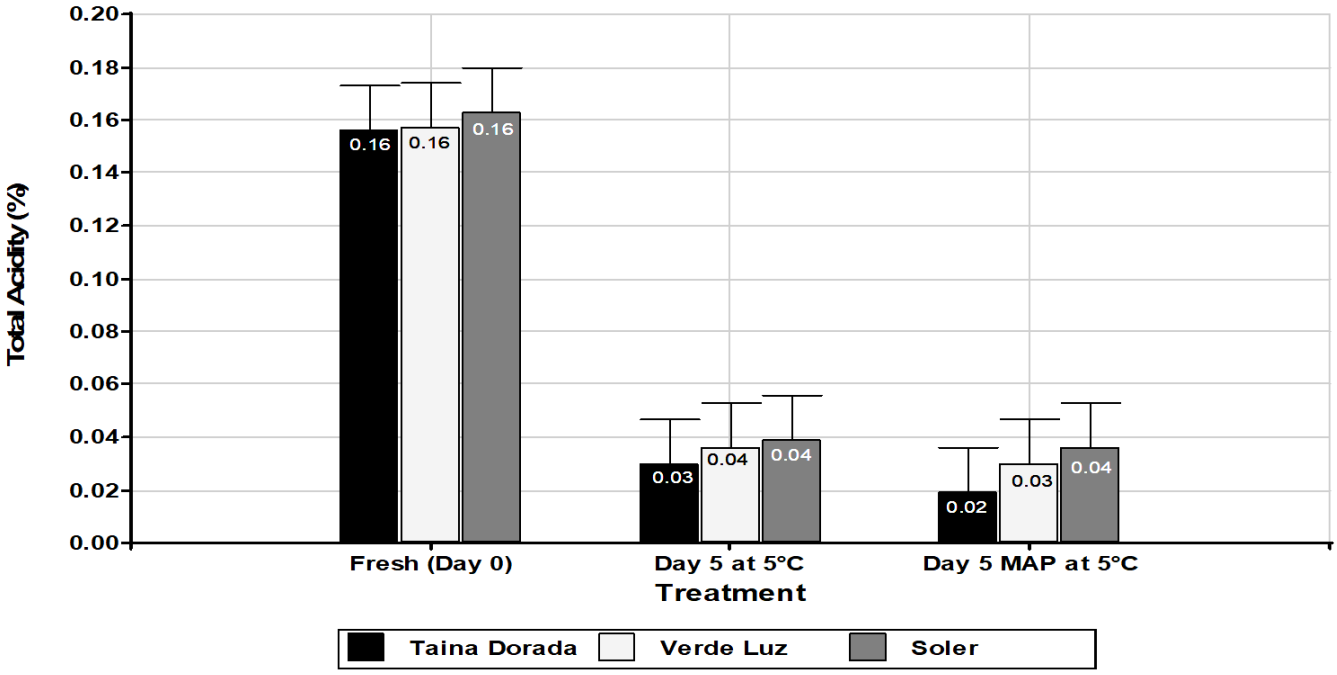

Figure 31: Mean percentage total acidity of fresh flowers of three genotypes of *Cucurbita* flowers stored at 5°C without modified atmosphere packaging (MAP) and with MAP for 5 days. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

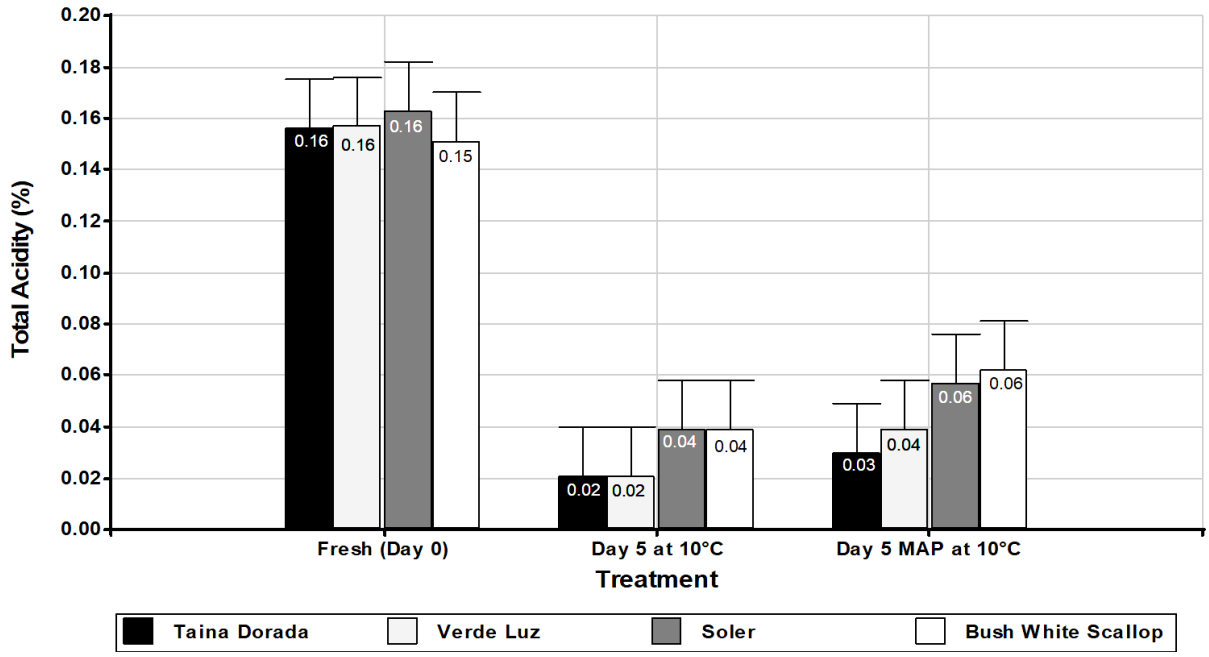

Figure 32: Mean percentage total acidity of fresh flowers of four genotypes of *Cucurbita* flowers stored at 10°C without modified atmosphere packaging (MAP) and with MAP for 5 days. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

For pH, the difference among genotypes varied depending on storage treatment (there was a significant genotype by storage treatment interaction (Table 15 and Table 16 in Appendix). There were no differences in pH among genotypes in freshly harvested pumpkin flowers stored at either 5°C (

Figure ***33***) or 10°C (

Figure ***34***). The pH in fresh flowers ranged from 6.67 to 6.77. Overall, pH increased from day 0 to day 5 of storage and there were significant differences in pH among genotypes at 5 days of storage. Flowers of Taína Dorada tended to have the highest pH, although this difference was not always significant, depending on storage temperature and packing type. Flowers of Soler were usually among those with the lowest pH, although again, these differences were not always significant.

Aquino-Bolaños et al. (2013) also observed an increase in pH during storage of *Cucurbita* flowers. In this present study, the increase in pH after storage was greater at 10˚C than at 5˚C. This can be due to the fact that at higher CO_2 production, pH will be greater (De Arruda, Grigoli, Ravási, Fátima, & Cechin, 2016). A study evaluated on the changes of pH in strawberries (*Fragaria x ananassa* Duch.) stored under controlled atmosphere demonstrated an increase in pH and decrease in total acidity under elevated CO_2

concentrations after storage. High concentrations of CO_2 could affect the organic acid metabolism, influencing the pH (Holcroft & Kader, 1999). Aquino-Bolaños et al. (2013) observed that an increase in pH is correlated to the decrease of total acidity (r = 0.85). In the current thesis study, pH increased and total acidity decreased after storage. At 5°C there was a strong correlation for MAP samples (r = 0.87) and low correlation for samples without MAP (r = 0.43) for pH and total acidity for each genotype and storage treatment. At 10°C, the correlation wasn't as strong as that for 5°C (r = 0.57 for samples without MAP and r = 0.63 for samples with MAP).

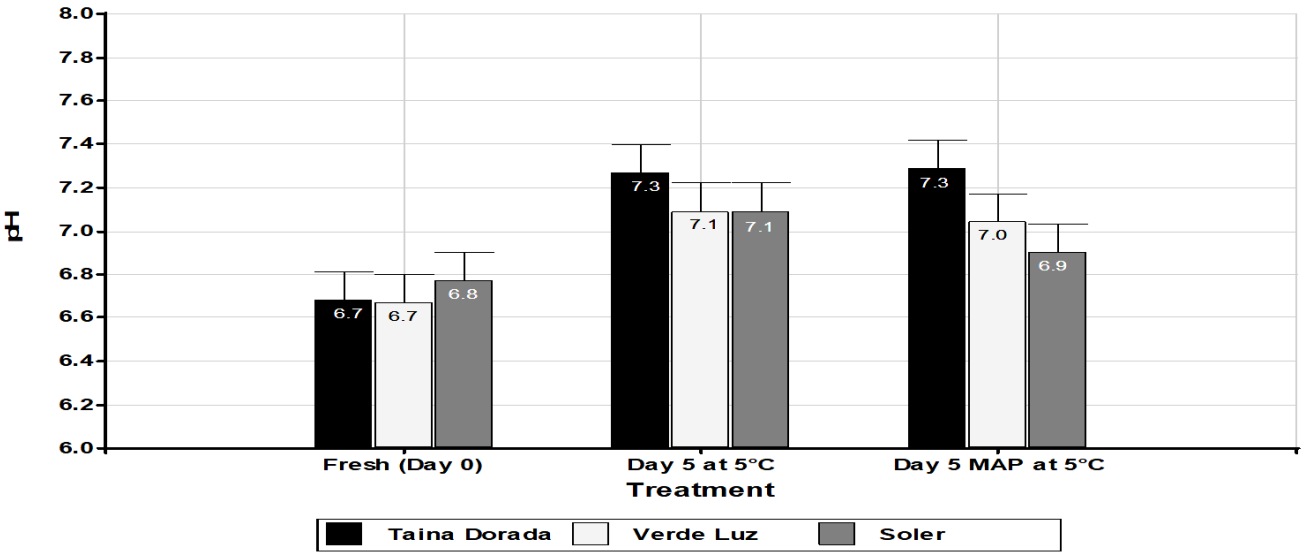

Figure 33: Mean pH of fresh flowers of three genotypes of *Cucurbita* flowers stored at 5°C without modified atmosphere packaging (MAP) and with MAP for 5 days. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

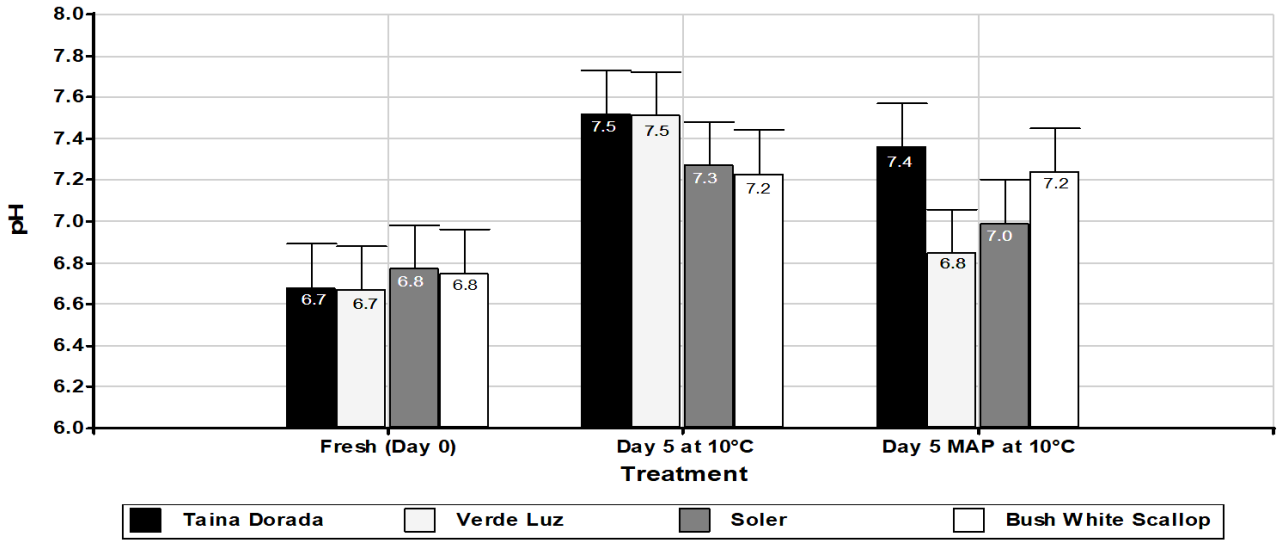

Figure 34: Mean pH of fresh flowers of four genotypes of *Cucurbita* flowers stored at 10°C without modified atmosphere packaging (MAP) and with MAP for 5 days. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

5.3.3. Color Parameters

Fresh pumpkin flower of genotypes had hue angles ranging from 69.04 to 72.56 (Table 6). A hue angle of 45 corresponds to orange and an angle of 90 corresponds to yellow. The range of hue angle values of fresh pumpkin flowers in this experiment was, therefore, very close to midway between orange and yellow (orange-yellow/yellow-orange or 67.5). Among the four genotypes, these tended towards a yellow-orange color (hue angle > 67.5). After 7 days of storage, flowers of all genotypes continued to have flower color close to 67.5 (midway between orange and yellow). On notable change was that flowers of Verde Luz had significantly more yellow color compared to the other genotypes.

Fresh pumpkin flowers had chroma ranging from 50.71 to 59.50. Chroma indicates intensity or saturation of the type of color. Early Prolific Straight-neck was the genotype that exhibited the most color saturation. After storage, chroma decreased, ranging from 34.34 to 54.20. Once again, Early Prolific Straightneck had the highest saturation being significantly different from the other genotypes. Taina Dorada had the least saturation out of all the genotypes.

Serocynksa et al. (2006), studying winter squash (*C. maxima*) flowers observed chroma values in flowers ranged from 42.2 to 85.7, a range considerably greater than in this thesis study. But like this thesis study, Serocynksa et al. (2006) also observed that, during storage, color parameters decreased for all

genotypes. Color loss is the first visible symptom of senescence in all horticultural crops (Ferrante, Incrocci, Maggini, Serra, & Tognoni, 2004).

Table 6*:* Mean hue angle and chroma of male flowers of four genotypes of *Cucurbita* before and after *7* days of storage with modified atmosphere packaging (MAP) at 10°C.

		Color							
		Before Storage				After Storage			
Genotype	*Cucurbita Species*	Hue Yellow		Chroma Yellow		Hue Yellow		Chroma Yellow	
Taina Dorada	*C. moschata*	71.53	a	50.90	b	70.34	bc	34.34	b
Verde Luz	*C. moschata*	72.56	a	50.71	b	76.29	a	37.16	b
Bush White Scallop	*C. pepo*	70.69	ab	55.88	ab	65.29	c	43.18	b
Early Prolific Straightneck	*C. pepo*	69.04	b	59.50	a	71.68	ab	54.20	a
LSD Tukey (0.05)		2.27		6.33		5.47		9.64	
F test (probability)		<0.0001		0.0008		0.0001		<0.0001	

Tukey's LSD = Tukey's least significant difference at the 0.05 level of probability
Within a column, means followed by a common letter are not different at the 0.05 level of probability.

5.3.4. Antioxidant Properties

5.3.4.1. Ascorbic Acid

For ascorbic acid (AA) content, there was a significant genotype by storage treatment interaction (Table 19 and Table 20 in Appendix). AA content in freshly harvested pumpkin flowers cultivars ranged from 16.14 to 16.15 mg/100 g FW and there were no differences among genotypes for storage at either 5°C (

Figure ***35***) nor at 10°C (

Figure ***36***). After storage, AA content decreased with a range of 3.0 to 8.06 mg/100 g FW. At 5°C, in general, there were few or no differences among genotypes within a storage treatment, but at 10°C the AA content was significantly greater with MAP compared to no MAP.

Aquino-Bolaños et al, (2013) observed AA content of approximately 16.51 mg/100 g FW for fresh flowers of *C. pepo.* The USDA Nutrient Database (2013) reports that fresh pumpkin flowers have 28 mg/100 g FW of AA. Grzeszczuk et al. (2016) evaluated AA content on 6 edible flowers species. The AA content ranged from 10.56 to 182.16 mg/100 g FW. Tahiroviç, et al., (2012) evaluated the AA content in 5 *Crataegus L.* edible flowers species and observed AA content ranging from 658.47 to 1108.00 mg/100 g DW (in comparison for the 269.17 mg/100 g DW of pumpkin flower).

After storage, Aquino-Bolaños et al. (2013) also observed a decrease in AA (to 8.17 mg/100 g FW). Das et al.(2010) also observed a decrease in AA content in a study evaluating Mahua flowers *Madhuca indica* syn *Bassia latifolia*). Loss of AA content after storage of minimally processed vegetables can be attributed to the fact that AA can be easily oxidized causing it to reduce during refrigerated storage (Howard et al., 1999).

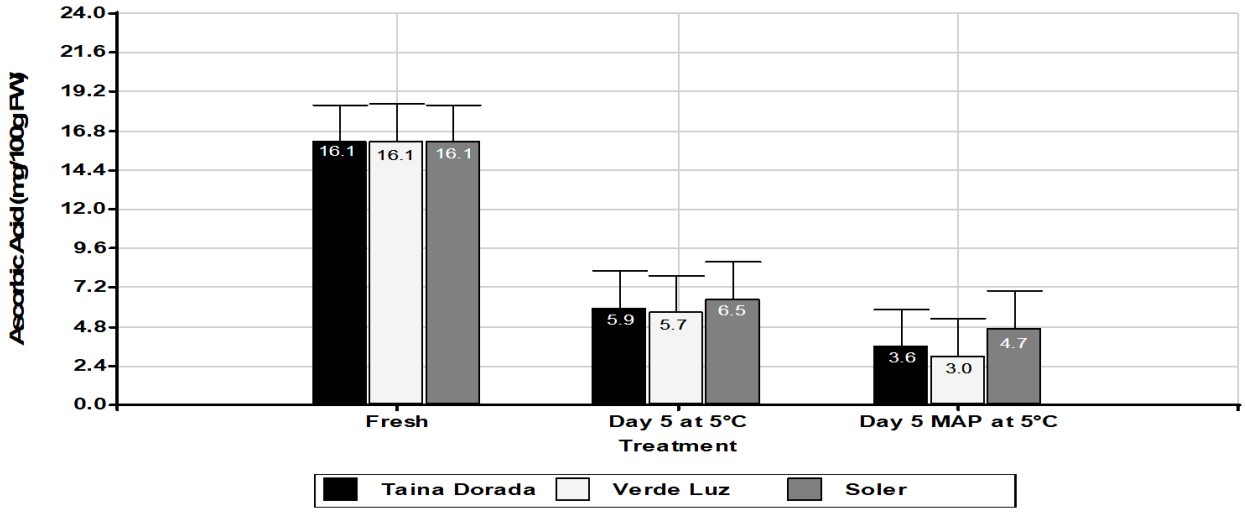

Figure 35: Mean ascorbic acid of fresh flowers of three genotypes of *Cucurbita* stored at 5°C without modified atmosphere packaging (MAP) and with MAP for 5 days. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

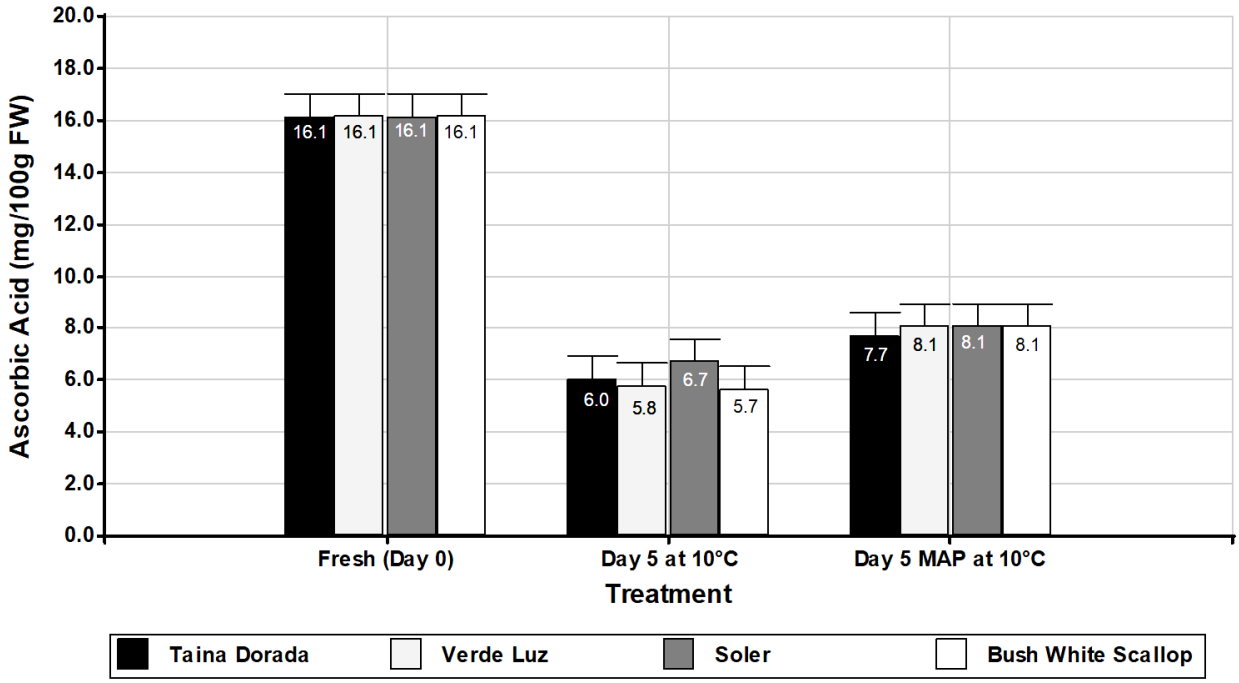

Figure 36: Mean ascorbic acid of fresh flowers of four genotypes of *Cucurbita* stored at 10°C without modified atmosphere packaging (MAP) and with MAP for 5 days. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

5.3.4.2. Beta-carotene

Initial beta-carotene content of the four genotypes of pumpkin flowers ranged from 10.38 to 14.12 mg/100 g FW. There was a strongly significant genotype by storage treatment interaction for beta-carotene of flowers stored at 10°C (Table 21 in Appendix) and a very weak interaction at 5°C (Table 22 in Appendix). At 5°C, there were no differences in beta-carotene among genotypes for fresh flowers, nor within each storage treatment (

Figure ***37***). At 5 days of storage beta-carotene increase in flowers of Taina Dorada, but not in flowers of Verde Luz and Soler. For flowers at 10°C, there were differences in beta-carotene among genotypes after 5 days in storage (

Figure ***38***), and the relative differences depended on the packaging treatment. In Taina Dorada, the levels of beta-carotene increased after storage and the increase was greater with MAP than without MAP. For the other genotypes (Verde Luz, Soler and Bush White Scallop) there was no difference in beta-carotene with or without MAP.

Serocyznka et al. (2006) observed beta-carotene levels for winter squash flowers ranging from 1.01 to 13.35 mg/100 g FW. The fruit of various pumpkin species had beta-carotene levels ranging from 0.06 to 7.40 mg/100 g FW (Murkovic, Mülleder, & Neunteufl, 2002). Results from this study shows that flowers have greater beta-carotene levels than pumpkin fruit.

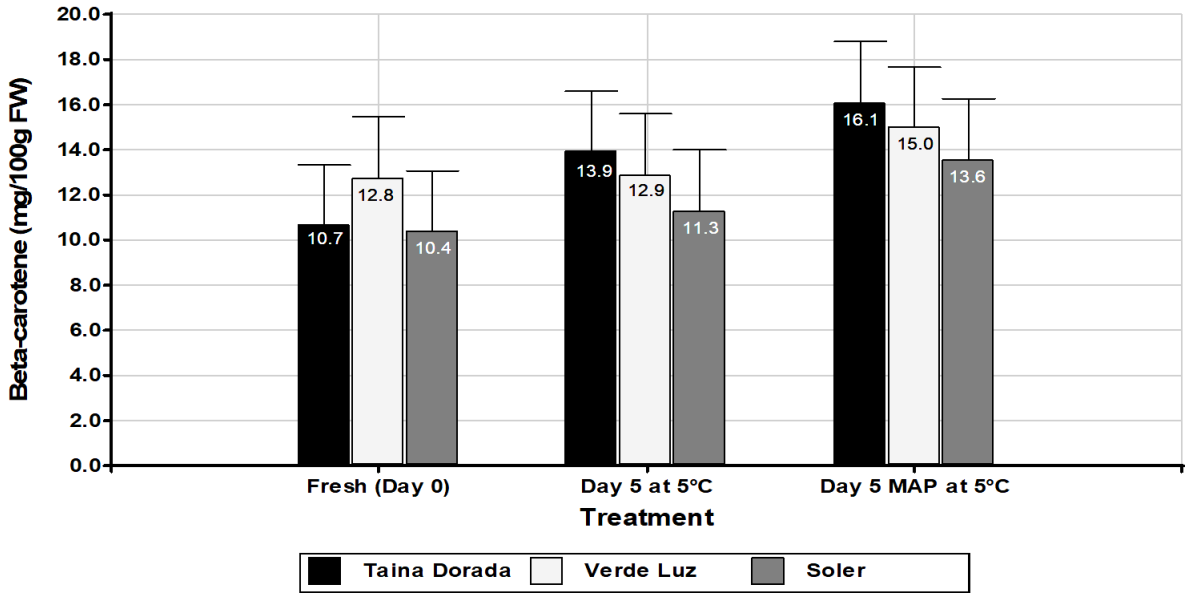

Figure 37: Mean beta-carotene of fresh flowers of three genotypes of *Cucurbita* stored at 5°C without modified atmosphere packaging (MAP) and with MAP for 5 days. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

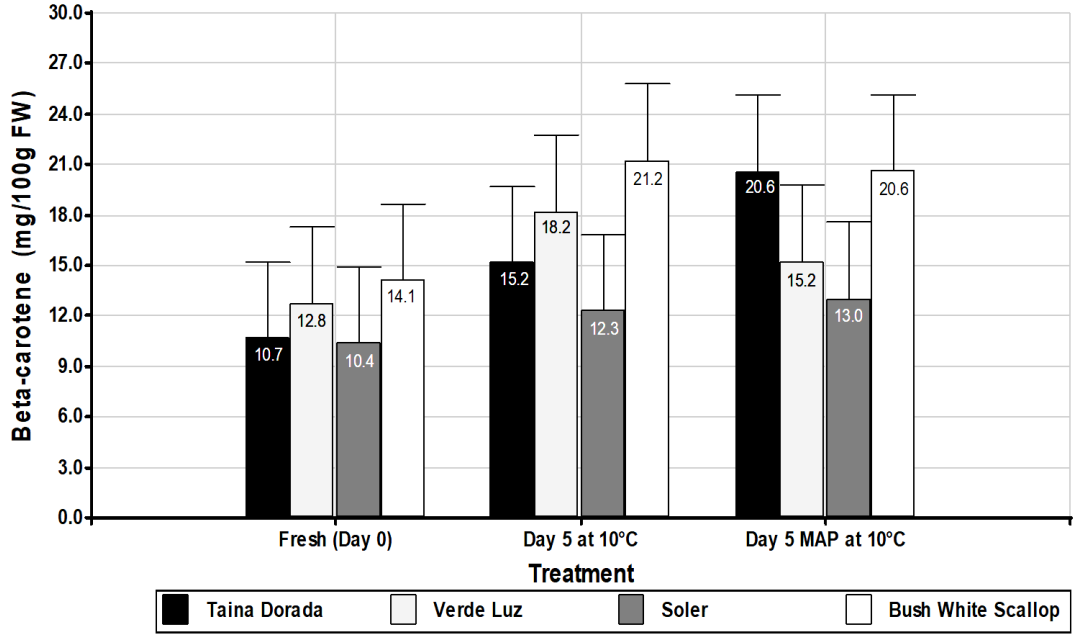

Figure 38: Mean beta-carotene of fresh flowers of three genotypes of *Cucurbita* stored at 10°C without modified atmosphere packaging (MAP) and with MAP for 5 days. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

In this study, several genotypes exhibited an increase in beta carotene levels during storage. During storage plant tissue loses water resulting in increased concentrations of pigments in these tissues. Carotenoids tend to be insoluble in water, meaning that they won't be affected by leaching losses during processing of fruits or vegetables (Meléndez, Vicario, & Heredia, 2004). Beta-carotene is related to the color pigment that is found in plants and gives yellow and orange fruits and vegetables their color (Ehrlich, 2015).

5.3.4.3. Phenolic Compounds

For total phenolic content, the difference among genotypes didn't vary depending on storage treatments (meaning there was no interaction for genotype per storage treatments) (Table 23 and Table 24 in Appendix). For this reason, the interpretation of results was based on main effects (Table 7). Total phenolic concentrations in fresh flowers ranged from 322.3 to 342.65 mg GAE/100 g FW. At 5°C, Soler had maintained the highest amount of total phenolic content compared with the other genotypes. The same behavior was observed at 10°C, where Soler was higher than the other genotypes but not significantly different from Taina Dorada and Verde Luz.

Compared to fresh flowers, total phenolics content for flowers without and with MAP stored at 5°C and 10°C for 5 days was reduced by fifty percent or more. At 10°C there were no differences in phenolics content between flowers stored with or without MAP. At 5°C storage with MAP resulted in lower phenolics content compared to storage without MAP.

Aquino-Bolaños et al. (2013) observed fresh flowers with an average of 334.60 mg GAE/100 g FW of phenolics. Li et al (2014) carried out a study of total phenolic content on 51 edible wild flowers and observed phenolic content ranging from 111 to 358.4 mg GAE/100g FW (comparable with this thesis). Chen et al., (2015) evaluated 23 edible flowers that were subjected to *in vitro* digestion to determine bioactive constituents. Phenolic content in these flowers ranged from 4.83 to 222.01 mg GAE/g DW. These results are also comparable with values obtained from this thesis study where phenolic content in pumpkin flowers ranged from 53.67 to 57.00 mg GAE/g DW.

During storage, there was a reduction in phenolic content. This is attributed to the fact that processing flowers affects all the availability of bioactive compounds. The phenolic compounds and other

antioxidants in food change during developmental processes and senescence. These processes activate molecular mechanisms and biochemical systems to counteract the free radicals (Cavaiuolo et al., 2013).

Table 7: Mean total phenolic content of male *Cucurbita* flowers of four genotypes at 5 days' post-harvest. Flowers packaged in plastic trays with and without (control) modified atmosphere packaging (MAP) at 5°C and 10°C.

	Total phenolic content (mg GAE/100 g FW)			
Effect	5°C		10°C	
Genotype (G)				
Bush White Scallop	---	---	182.78	c
Taina Dorada	199.02	b	191.33	b
Soler	210.66	a	199.45	b
Verde Luz	201.47	b	190.55	b
F Test (p value)	0.003		0.0001	
Tukey LSD (0.05)	7.67		7.51	
Treatment (T)				
Control	153.68	b	120.49	b
MAP	122.97	c	121.15	b
F test (p value)	<0.0001		<0.0001	
Tukey LSD (0.05)	7.67		5.88	
G x T				
F test (p value)	0.9285		0.3715	

Tukey-LSD: Least significant difference between means using the Tukey test at the 5% probability level. Within a column, means followed by a common letter are not different at the 5% probability level.

5.3.4.4. Antioxidant Activity

For the antioxidant activity of flowers, the difference of genotypes varied depending on storage treatment (significant genotype by storage treatment interaction) (Table 25 and Table 26 in Appendix). Fresh flowers had an antioxidant activity ranging from 38.78 to 94.21 µM TEAC/g FW (Figures 42 and 43). In fresh flowers, Verde Luz and Soler had antioxidant activities nearly twice as high as the other genotypes.

For flowers stored for 5 days at 5°C (

Figure ***39***), Soler and Verde Luz continued to have the highest amount of antioxidant activity when flowers were stored without MAP. There were no differences among genotypes at 5°C with MAP. For flowers stored for 5 days at 10°C (

Figure ***40***), Verde Luz maintained the highest antioxidant activity only in samples with MAP. Overall, samples stored at 5°C retained somewhat better antioxidant activity than samples stored at 10°C.

However, in general, storage resulted in a decrease of antioxidant activity for all storage (packaging) treatments. Flowers lost almost all their antioxidant activity during storage.

Chen and contributors (2015) evaluated the antioxidant activity on 23 edible flowers. These had DPPH values ranging from 21.14 to 599.43 µM TEAC/g DW. These values are lower than those obtained from this study where values ranged from 646.33 to 1570.17 µM TEAC/g DW. Li and contributors (2014) evaluated DPPH on 51 edible wild flowers. These had DPPH ranging from 0.23 to 175.39 µM TEAC/g FW. Navarro-González et al., (2015) studied antioxidant activity on three different species of edible flowers. These had an antioxidant activity ranging from 5.52 to 66.22 µM TEAC/g FW. The results in this thesis are comparable from the previously mentioned studies. Pumpkin flowers have a higher content of antioxidant activity than other edible flowers.

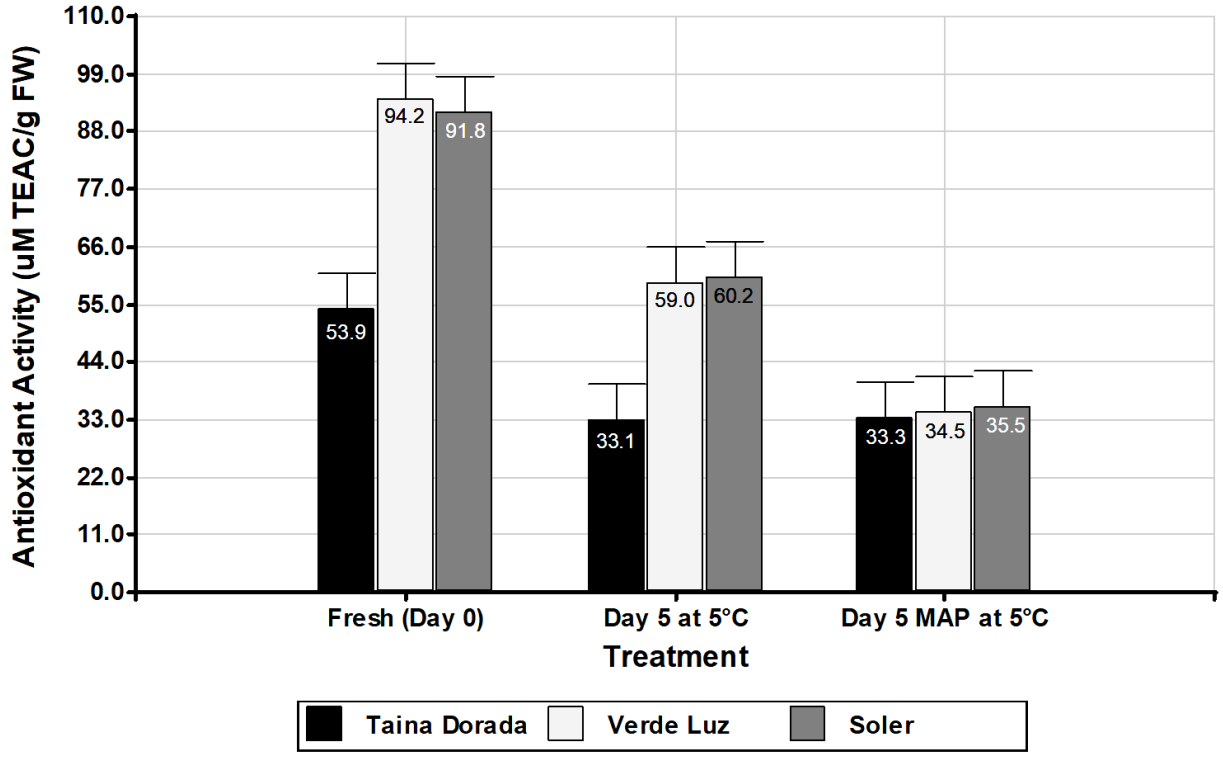

Figure 39: Mean antioxidant activity of fresh flowers of three genotypes of *Cucurbita* stored at 5°C without modified atmosphere packaging (MAP) and with MAP for 5 days. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

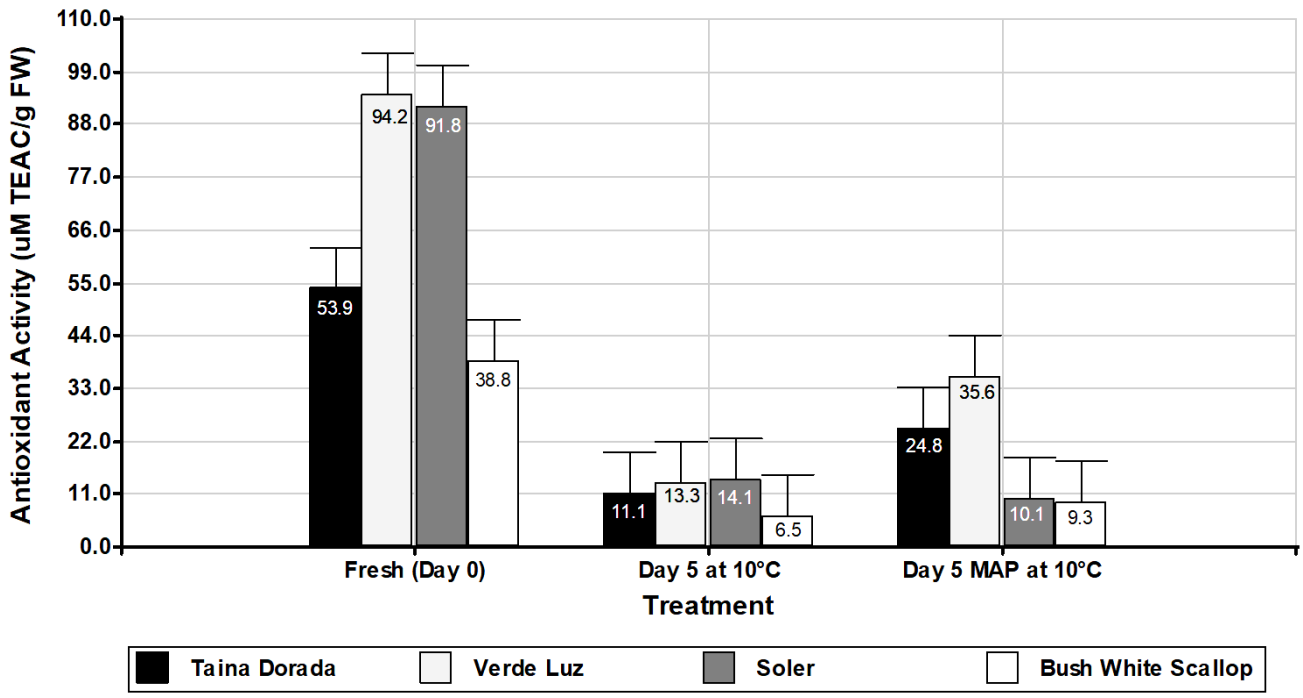

Figure 40: Mean antioxidant activity of fresh flowers of four genotypes of *Cucurbita* stored at 10°C without modified atmosphere packaging (MAP) and with MAP for 5 days. Vertical bars represent Tukey's least significant difference at 5% probability level for comparison of any two means.

5.4. Proximal Analysis and Micro Nutrients

Proximal analysis and micronutrients was evaluated on fresh flowers. The four genotypes of *Cucurbita* flowers had a moisture content ranging from 93.72 to 94.75% (Table 8), like that in the USDA Nutrient Database (2014). Protein content ranged from 1.37 to 1.91%, slightly higher than the 1.03% reported by the USDA. Total lipid content (fat) ranged from 0.09 to 0.15%, somewhat higher than the 0.07% stated by the USDA. Ash content ranged from 0.70 to 0.78%. This too is higher than what is stated by the USDA (ash content of 0.48%). The micronutrients present in pumpkin flowers were phosphorous, potassium, calcium, magnesium, iron, zinc, and manganese. The most prevalent mineral was potassium, ranging from 231.87 to 290.76 mg/100g FW. This value was higher than what is stated from the USDA database (173 mg/100g FW). Phosphorous ranged from 31.02 to 39.07 mg/100g FW and calcium ranged from 45.07 to 58.40 mg/100g, indicating that flowers of these four genotypes present a good source of these nutrients.

Table 8: Means of proximal analysis and mineral content of four genotypes of *Cucurbita* flowers.

	[1]Taina Dorada		[1]Verde Luz		[1]Bush White Scallop		[1]Soler		Tukey's-LSD
Proximal Analysis									
Moisture (%)	94.12	a	94.25	a	93.79	a	93.72	a	0.66
Ash (%)	0.70	b	0.73	ab	0.70	b	0.78	a	0.08
Fat (%)	0.15	a	0.13	a	0.09	b	0.09	b	0.03
Protein (%)	1.43	c	1.37	c	1.60	b	1.91	a	0.15
Carbohydrates (%) *	3.60		3.52		3.82		3.50		-
Minerals (mg/100g)									
P	31.02	b	31.26	b	39.47	a	33.91	b	5.19
K	231.87	b	278.11	a	278.59	a	290.76	a	13.76
Ca	52.22	a	53.80	a	45.07	a	58.40	a	23.09
Mg	23.76	a	20.50	a	24.18	a	25.96	a	5.18
Fe	1.06	b	1.20	b	1.07	b	2.07	a	0.33
Mn	0.21	a	0.13	c	0.24	a	0.17	b	0.03
Zn	0.16	ab	0.13	bc	0.11	c	0.17	a	0.03

Means with same letter are not significantly different at a probability level of $p > 0.05$ with Tukey test.
Tukey's-LSD = Tukey's Least Significance Difference at a 0.05 probability test.
* Carbohydrates was determined by difference with moisture, ash, protein and fat content.
[1]All genotypes are *C. moschata* except Bush White Scallop is *C. Pepo*.

5.5. Sensory Evaluations

Male pumpkin flowers were evaluated fresh and cooked, on three different days, places and participants. Evaluating the flowers in this way helped to have higher participation and give a real idea of possible consumer consumption. By pre-screening the participants this helped in viewing what types of consumers would eat the flowers if it were to be sold at a large-scale production. Fresh organic pumpkin flowers were evaluated on texture, flavor, and general acceptance. Crispness and crunchiness are part of textural attributes (Tunick et al., 2013). In this evaluation, texture was measured in terms of crunchiness using a total of 103 panelists. Crunchy texture is defined as a chew or press that makes a crushing noise (Merriam, Merriam, & Webster, 2008; Tunick et al., 2013). Flowers used to measure texture had a mean value of 7.63 corresponding to "like moderately" to "like extremely" on the hedonic scale (

Figure ***41***). It can be concluded that the texture had a great acceptability among consumers. A total of 93.2% of panelists rated acceptance of the texture of the flower on a range of 5 to 9 ("neither like nor dislike" to "like extremely") on the hedonic scale. From this, 88.5% of panelists rated acceptance of

texture ranging from 6 to 9 (“like moderately” to “like extremely”). Panelists who rated the flower with high scores mentioned that the flower had a texture like lettuce but was easier to swallow. They also said that it was better than regular vegetables that are normally consumed. Panelists that rated the texture of the flower with low scores mentioned that they would prefer the flower to be crunchier since it dissolved in the mouth like water.

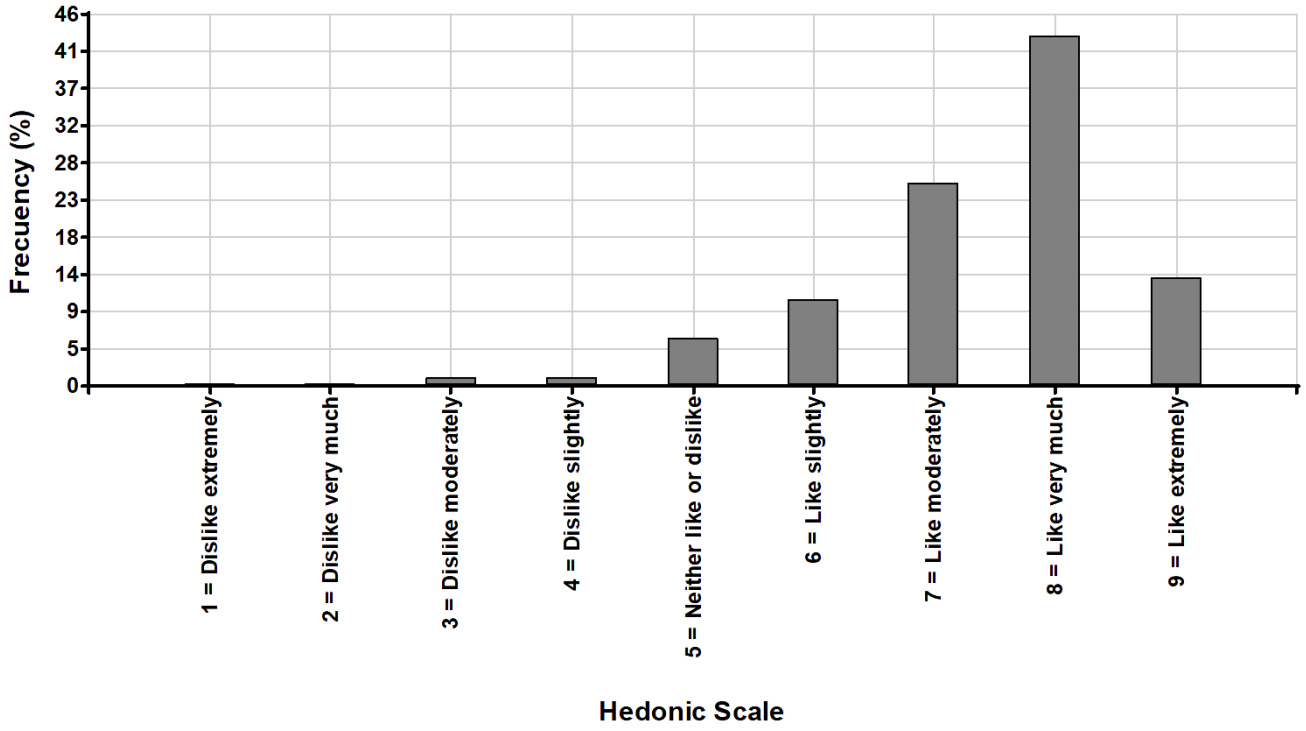

Figure 41: Overall acceptability of texture on fresh pumpkin flowers.

Taste was measured on fresh pumpkin flowers using a total of 112 panelists. The average score for taste was 7.23 which corresponds to “like moderately” to “like very much” on the hedonic scale (

Figure ***42***). A total of 98.2% of panelists rated taste acceptance for flowers from 5 to 9 on the hedonic scale. A total of 81.2% of panelists rated taste acceptance for flowers from 6 to 9 on the hedonic scale. The main factors that contributed to high rating scores for taste was that fresh flowers had an excellent flavor, were refreshing, and had a sweet/soft taste. Many mentioned that it was better than expected and they would love to consume pumpkin flowers as part of an ingredient of other dishes. On the other hand, some panelists also mentioned that the flower doesn’t have a taste and needs more flavor; they would gladly eat it with salad dressing.

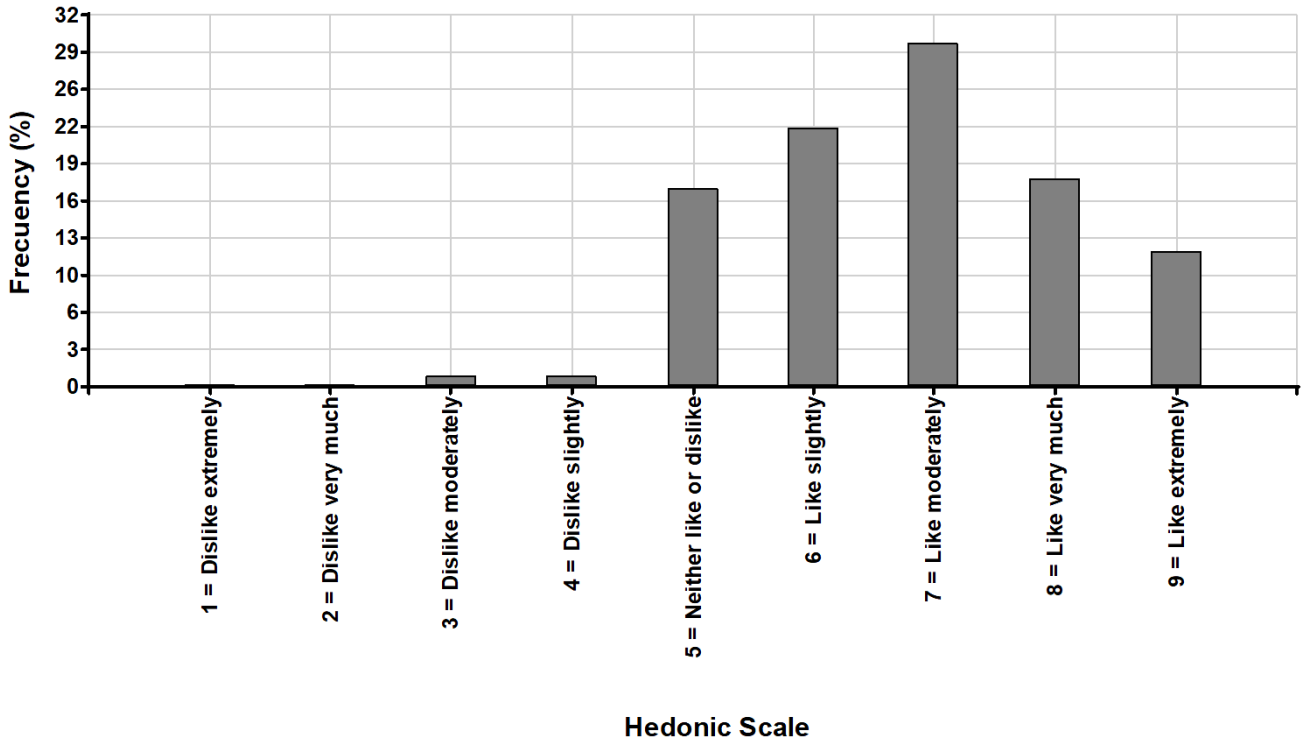

Figure 42: Overall acceptability of taste on fresh pumpkin flowers.

Overall acceptability of fresh pumpkin flowers was determined with a participation of 118 panelists. Fresh pumpkin flowers had an overall acceptability with a mean of 7.46 which ranges from "like moderately" to "like very much" (

Figure ***43***). A total of 95.7% of panelists rated acceptance for flowers in the range of 5 to 9 on the hedonic scale. Of those, 82.2% of panelists rated the flower from "like slightly" to "like moderately". Panelists commented that the flower was fresh, refreshing and appealing to the eye. They loved the taste, texture and color, making it an ingredient they would gladly eat with other meals. Many were interested in knowing its nutritional values. Other panelists said the flower by itself didn't have a good taste but they would eat it in salads. Also, in this sensory evaluation the panelists were asked if they would buy the product. Out of 118 panelists 82 of them said that they would buy this product if it was sold at a local market. This represents a total of 69.5%. On the other hand, 9.3% said they wouldn't buy this product because this is something they would consume at a gourmet restaurant and not part of their daily diet. A

total of 10.2% of panelists mentioned they were indecisive about buying the flowers since they would only buy it as an ingredient for other meals. The rest (10.9%) did not answer the question.

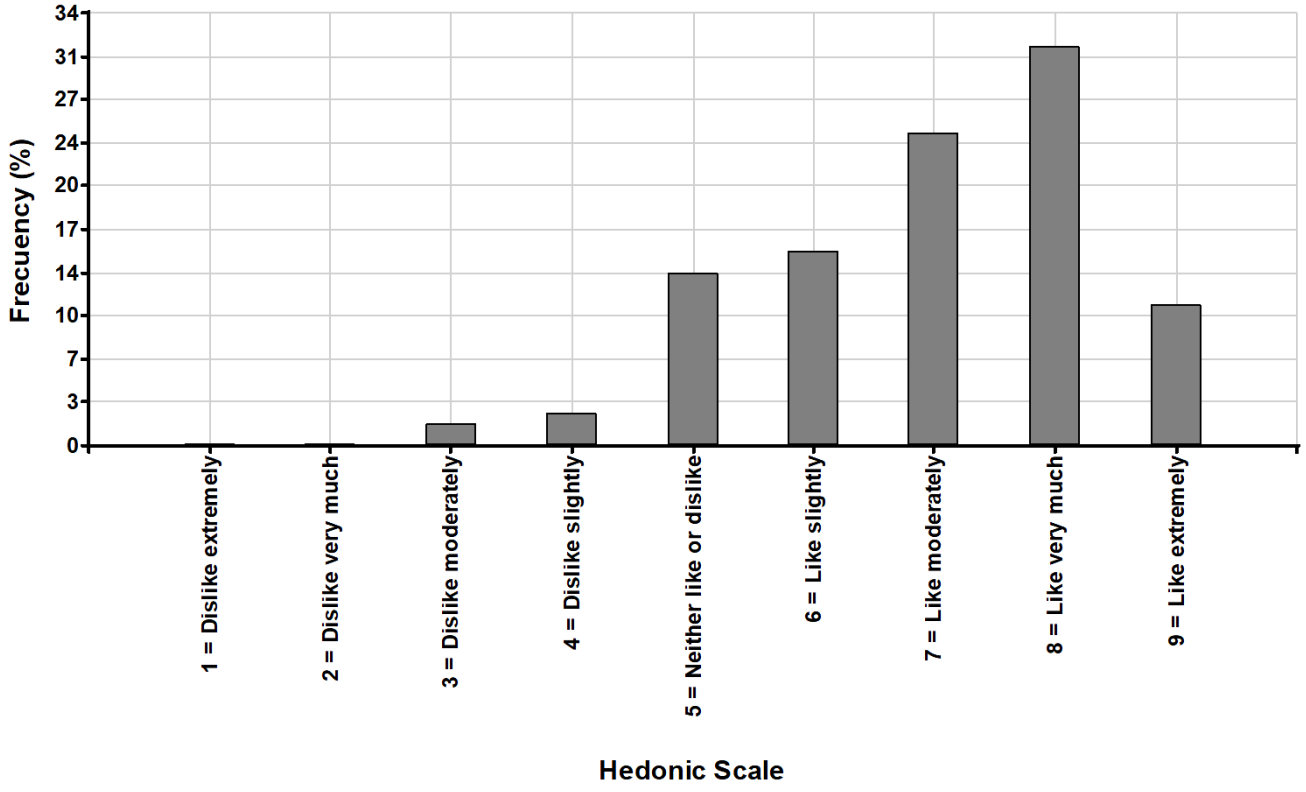

Figure 43: Overall acceptability of fresh pumpkin flowers.

Figure ***45*** shows the general appearance of minimally processed (canned,

Figure ***44***) *Cucurbita* flowers that were minimally processed had a mean of 6.96 on the hedonic scale, which corresponds to "like slightly" to "like moderately" on the hedonic scale. A total of 88.2% of panelists rated acceptance of minimally processed flowers from 5 to 9 on the hedonic scale. Of those, 62.2% of panelists rated the minimally processed pumpkin flower "like slightly "to "like moderately" (6 to 7 on hedonic scale). Panelists that scored minimally processed flowers from 7 to 9 included that these flowers had a good taste, texture, and it was appealable. They mentioned that it had good consistency and it was a good ingredient to be added in various dishes such as soups, and salads. Many compared it to the taste of different vegetables such as broccoli and said that they were surprised with the outcome. Panelists

that rated the flower with lower scores mentioned that the product needed more salt since it just tasted like "hot water". These comments could be taken into consideration to help improve the saline formulation.

Figure 44: Canned Pumpkin flowers for sensory evaluations.

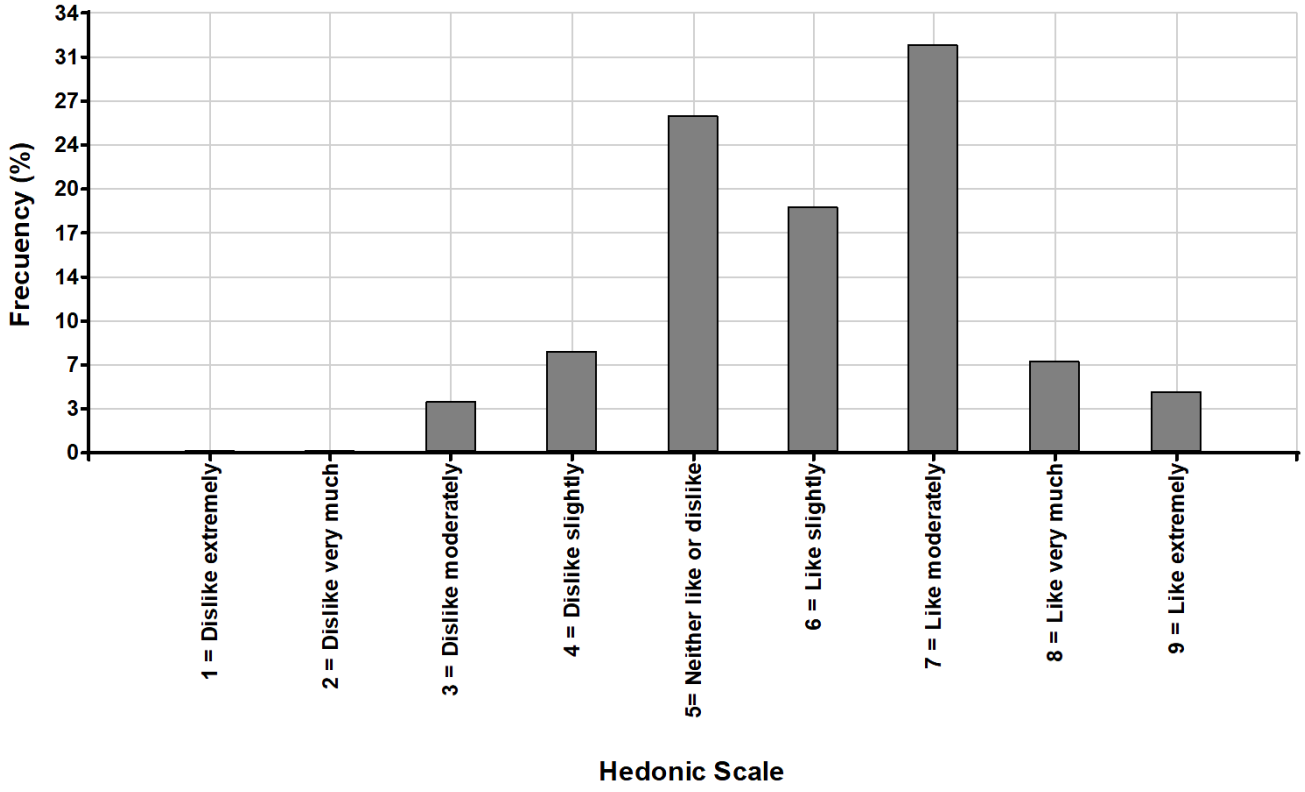

Figure 45: Overall acceptability of minimally processed pumpkin flowers.

6. Conclusion

The flowers of the four genotypes of *Cucurbita moschata* and *Cucurbita pepo* have nutritional value and good acceptance as both fresh and canned flowers making these genotypes ideal for consumption and commercialization. Verde Luz and Soler had the highest amount of phenolic content (332 and 342 mg GAE/100 g FW), and antioxidant activity (94.2 and 91.8 µM TEAC/g FW). Also, Bush White Scallop was a good source of beta-carotene content (14.1 mg/100 g FW) compared with all the other genotypes. Ascorbic acid content averaged of 16.15 mg/100 g FW with no significant difference between genotypes. Additionally, pumpkin flowers are a good source of calcium, phosphorous, and potassium that one can consume in their daily diet. Three genotypes, Taína Dorada, Verde Luz and Soler, retained acceptable visual appearance for 4 to 5 days of storage at 5 and 10°C. Flowers stored under MAP had a slightly better visual appearance than flowers stored without MAP. A fresh mixture of the three genotypes of *Cucurbita moschata* was rated "liked moderately" in texture, flavor, and general acceptance following the 9-point hedonic scale. Also, cooked flowers were rated as "liked moderately". Use of pumpkin flowers has the potential to contribute added value to Puerto Rico's gastronomy and agricultural economy.

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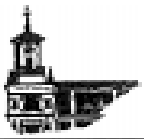
8. Appendix

8.1. Exemption letter from the Committee for the Protection of Human Subjects in Research.

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



November 4, 2015

Dr.Rosa Chavez- Jauregui
Crops & Agroenvironmental Sciences
RUM

Dear Dr. Chavez:

As a member of the Institutional Review Board of the University of Puerto Rico - Mayagüez Campus, I have considered the Review Application for your project titled *Cucurbita Flowers and Immature Fruit as New Food Products for Puerto Rico: Quality and Nutritional Assessment* (Protocol num. 20151101). After an evaluation of your protocol, I have determined that your research qualifies for an exempt approval according to Category 2 of 45.CFR.46.101(b)(2) and 46.101(b)(6).

Remember that 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. The IRB must likewise be notified immediately if any breach of confidentiality occurs.

We appreciate your commitment to uphold the highest standards of human research protections and remain.

Sincerely,

Dr. Rafael A. Bogle Martínez
President, Institutional Review Board (IRB)
University of Puerto Rico,
Mayagüez Campus
Office: Celis 108
Tel.: (787) 832-4040 Ext. 6277
Web Page: http://www.uprm.edu/cpshi/

Telephone: (787) 832 - 4040 x 6277, 3807, 3808 – **Fax:** (787) 831-2005 –**Webpage:** www.uprm.edu/cpshi
Email: cpshi@uprm.edu

8.2. Factorial design of pumpkin flowers

Model design for this experiment- Factorial design 4 x 2

$$\mathbf{Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}}$$

Where μ is the mean, *i* equals the levels of storage treatment, *j* equals the levels of genotypes, *k* is the repetitions, α_i is the effect of storage treatment, β_j is the effect of genotype per storage treatment, and ε is the experimental error.

Hypothesis

Interaction

$H_o = \alpha\beta$ (no interaction)

$H_a \neq \alpha\beta$ (interaction)

Effect of Storage Treatment

H_o: $\alpha = 0$ (no principal effect)

H_a: $\alpha \neq 0$

Effect of Genotype

H_o: $\alpha = 0$ (no principal effect)

H_a: $\alpha \neq 0$

8.3. Yield Performance

Table 9: Mean of total number of flowers, average number of flowers per day, average weight (g) and average length (cm) of five genotypes of *Cucurbita* male flowers.

Total number of flowers

Variable	N	R^2	R^2 Aj	CV
Total number	197	0.20	0.19	52.65

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model	2061.32	4	515.33	12.24	<0.0001
Genotype	2061.32	4	515.33	12.24	<0.0001
Error	8085.89	192	42.11		
Total	10147.21	196			

Test: Tukey Alfa=0.05 DMS=4.05042

Error: 42.1140 gl: 192

Genotype	Means	n	E.E.			
Bush White Scallop	16.11	47	0.95	A		
Verde Luz	13.68	31	1.17	A	B	
Taina Dorada	13.51	37	1.07	A	B	
E1101-1	11.77	35	1.10		B	
Early Pacific Straightneck..	7.13	47	0.95			C

Means with a same letter in common are not significantly different (p > 0.05)

Average number per plant per day

Variable	N	R^2	R^2 Aj	CV
Num_PlTA	197	0.20	0.19	52.65

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	128.83	4	32.21	12.24	<0.0001
Genotype	128.83	4	32.21	12.24	<0.0001
Error	505.37	192	2.63		
Total	634.20	196			

Test: Tukey Alfa=0.05 DMS=1.01260

Error: 2.6321 gl: 192

Genotype	Means	n	E.E.			
Bush White Scallop	4.03	47	0.24	A		
Verde Luz	3.42	31	0.29	A	B	
Taina Dorada	3.38	37	0.27	A	B	
E1101-1	2.94	35	0.27		B	
Early Pacific Straightneck..	1.78	47	0.24			C

Means with a same letter in common are not significantly (p > 0.05)

Average weight of flowers (g)

Variable	N	R^2	R^2 Aj	CV
weight (g)	197	0.82	0.81	21.35

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	1299.51	4	324.88	213.16	<0.0001
Genotype	1299.51	4	324.88	213.16	<0.0001
Error	292.62	192	1.52		
Total	1592.13	196			

Test: Tukey Alfa=0.05 DMS=0.77053

Error: 1.5241 gl: 192

Genotype	Means	n	E.E.			
Verde Luz	8.80	31	0.22	A		
Taina Dorada	8.05	37	0.20	A	B	
E1101-1	7.87	35	0.21		B	
Early Pacific Straightneck..	3.29	47	0.18			C
Bush White Scallop	2.93	47	0.18			C

Means with a same letter in common are not significantly (p > 0.05)

Length (cm)

Variable	N	R^2	R^2 Aj	CV
Length (cm)	197	0.82	0.81	8.64

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	689.44	4	172.36	213.07	<0.0001
Genotype	689.44	4	172.36	213.07	<0.0001
Error	155.31	192	0.81		
Total	844.76	196			

Test: Tukey Alfa=0.05 DMS=0.56136

Error: 0.8089 gl: 192

Genotype	Medias	n	E.E.			
E1101-1	12.71	35	0.15	A		
Verde Luz	12.47	31	0.16	A		
Taina Dorada	11.33	37	0.15		B	
Bush White Scallop	8.54	47	0.13			C
Early Pacific Straightneck..	8.47	47	0.13			C

Means with a same letter in common are not significantly (p > 0.05)

Table 10: Mean of total number of flowers, and average number of flowers per day of five genotypes of *Cucurbita* female flowers.

Total number of female flowers

Variable	N	R^2	R^2 Aj	CV
Num-Total	159	0.23	0.21	62.99

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	169.71	4	42.43	11.26	<0.0001
Genotype	169.71	4	42.43	11.26	<0.0001
Error	580.23	154	3.77		
Total	749.94	158			

Test: Tukey Alfa=0.05 DMS=1.56815

Error: 3.7677 gl: 154

Genotype	Means	n	E.E.		
EPS	4.42	52	0.27	A	
BWS	3.04	45	0.29	A	B
TD	2.16	25	0.39		B
E1101-1	1.89	27	0.37		B
VL	1.80	10	0.61		B

Means with a same letter in common are not significantly (p > 0.05)

Average number of female flower per plant

Variable	N	R^2	R^2 Aj	CV
Num_Av_Plant	159	0.23	0.21	62.99

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	10.61	4	2.65	11.26	<0.0001
Genotype	10.61	4	2.65	11.26	<0.0001
Error	36.26	154	0.24		
Total	46.87	158			

Test: Tukey Alfa=0.05 DMS=0.39204

Error: 0.2355 gl: 154

Genotypes	Means	n	E.E.		
EPS	1.11	52	0.07	A	
BWS	0.76	45	0.07	A	B
TD	0.54	25	0.10		B
E1101-1	0.47	27	0.09		B
VL	0.45	10	0.15		B

Means with a same letter in common are not significantly (p > 0.05)

8.4. Weight loss, CO_2 and O_2 gas composition during storage

Table 11: Weight loss, CO_2 and O_2 gas composition of four genotypes of *Cucurbita* during storage at 10°C

CO2 (%)

Variable	N	R²	R² Aj	CV
CO2 (%)	48	0.97	0.95	14.10

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	2632.28	17	154.84	56.01	<0.0001
TRT	2278.87	3	759.62	274.76	<0.0001
Genotype	80.60	3	26.87	9.72	0.0001
Run	0.38	2	0.19	0.07	0.9343
TRT*Genotype	272.43	9	30.27	10.95	<0.0001
Error	82.94	30	2.76		
Total	2715.22	47			

Test: Tukey Alfa=0.05 DMS=1.44925

Error: 2.7647 gl: 30

Run	Means	n	E.E.	
3	11.86	16	0.42	A
1	11.84	16	0.42	A
2	11.66	16	0.42	A

Test: Tukey Alfa=0.05 DMS=5.06030

Error: 2.7647 gl: 30

TRT	Genotype	Meand	n	E.E.						
Stored 10 Control	Soler	23.83	3	0.96	A					
Stored 10 Control	VL	19.74	3	0.96	A	B				
Stored 10 MAP	Pepo	19.34	3	0.96	A	B				
Stored 10 MAP	VL	18.62	3	0.96		B				
Stored 10 MAP	Soler	18.01	3	0.96		B				
Stored 10 MAP	TD	15.71	3	0.96		B	C			
Stored 10 Control	TD	15.32	3	0.96		B	C			
Fresh MAP	Pepo	11.64	3	0.96			C	D		
Fresh MAP	TD	11.49	3	0.96			C	D		
Fresh MAP	Soler	11.28	3	0.96			C	D		
Fresh MAP	VL	11.23	3	0.96			C	D		
Stored 10 Control	Pepo	9.70	3	0.96				D		
Fresh Control	VL	0.86	3	0.96						E
Fresh Control	Soler	0.75	3	0.96						E
Fresh Control	TD	0.57	3	0.96						E
Fresh Control	Pepo	0.53	3	0.96						E

Means with the same letter are not significantly different with 0.05 probability level Tukey's test

TD = Taina Dorada

VL = Verde Luz

Pepo = Bush White Scallop

O2 (%)

Variable	N	R^2	R^2 Aj	CV
O2 (%)	48	0.98	0.96	14.77

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	2510.05	17	147.65	75.30	<0.0001
TRT	2226.29	3	742.10	378.47	<0.0001
Genotype	85.28	3	28.43	14.50	<0.0001
Run	0.16	2	0.08	0.04	0.9608
TRT*Genotype	198.33	9	22.04	11.24	<0.0001
Error	58.82	30	1.96		
Total	2568.88	47			

Test: Tukey Alfa=0.05 DMS=1.22048

Error: 1.9608 gl: 30

Run	Means	n	E.E.	
1	9.55	16	0.35	A
2	9.48	16	0.35	A
3	9.41	16	0.35	A

Test: Tukey Alfa=0.05 DMS=4.26153

Error: 1.9608 gl: 30

TRT	Genotype	Means	n	E.E.				
Fresh Control	Pepo	21.00	3	0.81	A			
Fresh Control	Soler	20.88	3	0.81	A			
Fresh Control	TD	20.81	3	0.81	A			
Fresh Control	VL	20.69	3	0.81	A			
Stored 10 Control	Pepo	13.46	3	0.81		B		
Stored 10 Control	TD	8.38	3	0.81			C	
Fresh MAP	Soler	7.66	3	0.81			C	
Fresh MAP	Pepo	7.60	3	0.81			C	
Fresh MAP	TD	7.52	3	0.81			C	
Fresh MAP	VL	7.43	3	0.81			C	
Stored 10 MAP	TD	4.85	3	0.81			C	D
Stored 10 Control	VL	4.46	3	0.81			C	D
Stored 10 MAP	Pepo	2.47	3	0.81				D
Stored 10 MAP	Soler	2.02	3	0.81				D
Stored 10 MAP	VL	1.63	3	0.81				D
Stored 10 Control	Soler	0.81	3	0.81				D

Means with the same letter are not significantly different with 0.05 probability level Tukey's test

TD = Taina Dorada

VL = Verde Luz

Pepo = Bush White Scallop

Weight loss

Variable	N	R^2	R^2 Aj	CV
Weight loss (%)	25	0.31	0.13	39.96

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	284.80	5	56.96	1.71	0.1803
Genotype	226.19	2	113.09	3.40	0.0547
TRT	22.01	1	22.01	0.66	0.4261
Genotype*TRT	5.63	2	2.82	0.08	0.9192
Error	632.17	19	33.27		
Total	916.97	24			

Test: Tukey Alfa=0.05 DMS=8.94425

Error: 33.2723 gl: 19

Genotype	Means	n	E.E.		
Pepo	17.73	4	2.88	A	
TD	14.96	17	1.42	A	B
VL	7.74	4	2.88		B

Test: Tukey Alfa=0.05 DMS=4.86435

Error: 33.2723 gl: 19

Treatment	Means	n	E.E.	
Day 3 Stored Control	14.65	14	2.02	A
Day 3 Stored MAP	12.31	11	2.06	A

Test: Tukey Alfa=0.05 DMS=15.75883

Error: 33.2723 gl: 19

Genotype	Treatment	Means	n	E.E.	
Pepo	Day 3 Stored Control	19.40	2	4.08	A
TD	Day 3 Stored Control	16.52	10	1.82	A
Pepo	Day 3 Stored MAP	16.05	2	4.08	A
TD	Day 3 Stored MAP	13.41	7	2.18	A
VL	Day 3 Stored Control	8.03	2	4.08	A
VL	Day 3 Stored MAP	7.46	2	4.08	A

Means with the same letter are not significantly different with 0.05 probability level Tukey's test
TD = Taina Dorada
VL = Verde Luz
Pepo = Bush White Scallop

Table 12: Weight loss, CO_2 and O_2 gas composition of four genotypes of *Cucurbita* during storage at 5°C

CO2 (%)

Variable	N	R^2	R^2 Aj	CV
CO2 (%)	36	0.97	0.95	13.67

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	1885.59	13	145.05	50.86	<0.0001
TRT	1770.57	3	590.19	206.95	<0.0001
Run	19.70	2	9.85	3.45	0.0496
Genotype	29.04	2	14.52	5.09	0.0152
TRT*Genotype	66.28	6	11.05	3.87	0.0087
Error	62.74	22	2.85		
Total	1948.33	35			

Test: Tukey Alfa=0.05 DMS=1.73190

Error: 2.8519 gl: 22

Run	Means	n	E.E.		
1	13.34	12	0.49	A	
2	12.14	12	0.49	A	B
3	11.56	12	0.49		B

Test: Tukey Alfa=0.05 DMS=5.01576

Error: 2.8519 gl: 22

TRT	Genotype	Means	n	E.E.						
Stored 5 MAP	Soler	20.77	3	0.98	A					
Stored 5 Control	Soler	20.69	3	0.98	A					
Stored 5 MAP	TD	18.56	3	0.98	A	B				
Stored 5 Control	VL	17.85	3	0.98	A	B	C			
Stored 5 MAP	VL	15.16	3	0.98		B	C	D		
Stored 5 Control	TD	15.14	3	0.98		B	C	D		
Fresh MAP	TD	12.93	3	0.98			C	D		
Fresh MAP	VL	12.42	3	0.98				D		
Fresh MAP	Soler	12.14	3	0.98				D		
Fresh Control	VL	0.90	3	0.98						E
Fresh Control	Soler	0.83	3	0.98						E
Fresh Control	TD	0.82	3	0.98						E

Means with the same letter are not significantly different with 0.05 probability level Tukey's test

TD = Taina Dorada

VL = Verde Luz

O2 (%)

Variable	N	R^2	R^2 Aj	CV
O2 (%)	36	0.95	0.92	21.68

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	1700.49	13	130.81	30.20	<0.0001
TRT	1584.25	3	528.08	121.94	<0.0001
Run	14.38	2	7.19	1.66	0.2129
Genotype	29.10	2	14.55	3.36	0.0533
TRT*Genotype	72.75	6	12.13	2.80	0.0353
Error	95.27	22	4.33		
Total	1795.77	35			

Test: Tukey Alfa=0.05 DMS=2.13419

Error: 4.3307 gl: 22

Run	Means	n	E.E.	
3	10.14	12	0.60	A
2	9.94	12	0.60	A
1	8.71	12	0.60	A

Test: Tukey Alfa=0.05 DMS=6.18081

Error: 4.3307 gl: 22

TRT	Genotype	Means	n	E.E.			
Fresh Control	TD	21.01	3	1.20	A		
Fresh Control	VL	20.91	3	1.20	A		
Fresh Control	Soler	20.87	3	1.20	A		
Stored 5 Control	TD	9.53	3	1.20		B	
Stored 5 MAP	VL	7.21	3	1.20		B	C
Fresh MAP	VL	7.14	3	1.20		B	C
Fresh MAP	Soler	7.09	3	1.20		B	C
Fresh MAP	TD	6.64	3	1.20		B	C
Stored 5 Control	VL	5.60	3	1.20		B	C
Stored 5 Control	Soler	4.24	3	1.20		B	C
Stored 5 MAP	TD	3.83	3	1.20		B	C
Stored 5 MAP	Soler	1.11	3	1.20			C

Means with the same letter are not significantly different with 0.05 probability level Tukey's test

TD = Taina Dorada

VL = Verde Luz

Weight loss

Variable	N	R^2	R^2 Aj	CV
Weight loss (%)	24	0.77	0.71	18.14

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	230.61	5	46.12	12.30	<0.0001
Genotype	226.50	2	113.25	30.20	<0.0001
TRT	0.38	1	0.38	0.10	0.7547
Genotype*TRT	3.74	2	1.87	0.50	0.6154
Error	67.49	18	3.75		
Total	298.11	23			

Test: Tukey Alfa=0.05 DMS=2.47102

Error: 3.7497 gl: 18

Genotype	Means	n	E.E.		
Pepo	14.79	8	0.68	A	
TD	9.82	8	0.68		B
VL	7.41	8	0.68		B

Test: Tukey Alfa=0.05 DMS=1.66086

Error: 3.7497 gl: 18

TRT	Means	n	E.E.	
Stored 5 Control	10.80	12	0.56	A
Stored 5 MAP	10.55	12	0.56	A

Test: Tukey Alfa=0.05 DMS=4.35153

Error: 3.7497 gl: 18

Genotype	TRT	Means	n	E.E.		
Pepo	Stored 5 Control	14.81	4	0.97	A	
Pepo	Stored 5 MAP	14.77	4	0.97	A	
TD	Stored 5 MAP	10.12	4	0.97		B
TD	Stored 5 Control	9.53	4	0.97		B
VL	Stored 5 Control	8.06	4	0.97		B
VL	Stored 5 MAP	6.76	4	0.97		B

Means with the same letter are not significantly different with 0.05 probability level Tukey's test
TD = Taina Dorada
VL = Verde Luz
Pepo = Bush White Scallop

8.5. Physicochemical measurements

Table 13:Statistical analysis of the factorial design for the effect of Brix on storage treatment for four genotypes of *Cucurbita* at 10°C

Brix

Variable	N	R^2	R^2 Aj	CV
Brix	36	0.97	0.95	5.82

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Modelo.	18.25	13	1.40	52.14	<0.0001
Run	0.08	2	0.04	1.48	0.2494
Genotype	5.50	3	1.83	68.03	<0.0001
TRT	10.81	2	5.40	200.66	<0.0001
Genotype*TRT	1.87	6	0.31	11.57	<0.0001
Error	0.59	22	0.03		
Total	18.84	35			

Test: Tukey Alfa=0.05 DMS=0.16829

Error: 0.0269 gl: 22

Run	Means	n	E.E.	
3	2.88	12	0.05	A
1	2.81	12	0.05	A
2	2.77	12	0.05	A

Test: Tukey Alfa=0.05 DMS=0.48738

Error: 0.0269 gl: 22

Genotype	TRT	Means	n	E.E.					
Soler	Fresh	4.08	3	0.09	A				
Pepo	Fresh	3.55	3	0.09		B			
VL	Fresh	3.40	3	0.09		B			
TD	Fresh	3.33	3	0.09		B	C		
Soler	Stored 10 Control	2.87	3	0.09			C	D	
TD	Stored 10 MAP	2.83	3	0.09				D	
Soler	Stored 10 MAP	2.80	3	0.09				D	
Pepo	Stored 10 MAP	2.76	3	0.09				D	
TD	Stored 10 Control	2.52	3	0.09				D	
Pepo	Stored 10 Control	2.51	3	0.09				D	
VL	Stored 10 Control	1.70	3	0.09					E
VL	Stored 10 MAP	1.44	3	0.09					E

Means with the same letter are not significantly different with 0.05 probability level Tukey's test

TD = Taina Dorada

VL = Verde Luz

Pepo = Bush White Scallop

Table 14: Statistical analysis of the factorial design for the effect of Brix on storage treatment for four genotypes of *Cucurbita* at 5°C

Brix

Variable	N	R^2	R^2 Aj	CV
Brix	27	0.99	0.98	3.45

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	13.69	10	1.37	144.56	<0.0001
Run	0.06	2	0.03	3.30	0.0633
Genotype	3.82	2	1.91	201.60	<0.0001
TRT	8.36	2	4.18	441.13	<0.0001
Genotype*TRT	1.45	4	0.36	38.38	<0.0001
Error	0.15	16	0.01		
Total	13.85	26			

Test: Tukey Alfa=0.05 DMS=0.11839

Error: 0.0095 gl: 16

Run	Means	n	E.E.	
3	2.89	9	0.03	A
1	2.79	9	0.03	A
2	2.78	9	0.03	A

Test: Tukey Alfa=0.05 DMS=0.28271

Error: 0.0095 gl: 16

Genotype	TRT	Means	n	E.E.					
Soler	Fresh	4.07	3	0.06	A				
VL	Fresh	3.40	3	0.06		B			
TD	Fresh	3.34	3	0.06		B			
Soler	Stored 5 MAP	3.15	3	0.06		B			
Soler	Stored 5 Control	2.66	3	0.06			C		
TD	Stored 5 Control	2.53	3	0.06			C		
TD	Stored 5 MAP	2.51	3	0.06			C		
VL	Stored 5 Control	2.17	3	0.06				D	
VL	Stored 5 MAP	1.55	3	0.06					E

Means with the same letter are not significantly different with 0.05 probability level Tukey's test

TD = Taina Dorada

VL = Verde Luz

Table 15:Statistical analysis of the factorial design for the effect of pH on storage treatment for four genotypes of *Cucurbita* at 10°C

pH

Variable	N	R^2	R^2 Aj	CV
pH	36	0.97	0.95	1.00

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	3.40	13	0.26	51.78	<0.0001
Run	6.0E-04	2	3.0E-04	0.06	0.9425
Genotype	0.19	3	0.06	12.37	0.0001
TRT	2.68	2	1.34	265.41	<0.0001
Genotype*TRT	0.53	6	0.09	17.51	<0.0001
Error	0.11	22	0.01		
Total	3.51	35			

Test: Tukey Alfa=0.05 DMS=0.07287

Error: 0.0050 gl: 22

Run	Means	n	E.E.	
3	7.08	12	0.02	A
2	7.07	12	0.02	A
1	7.07	12	0.02	A

Test: Tukey Alfa=0.05 DMS=0.21103

Error: 0.0050 gl: 22

Genotype	TRT	Means	n	E.E.				
TD	Stored 10 Control	7.52	3	0.04	A			
VL	Stored 10 Control	7.51	3	0.04	A			
TD	Stored 10 MAP	7.36	3	0.04	A	B		
Soler	Stored 10 Control	7.27	3	0.04		B		
Pepo	Stored 10 MAP	7.24	3	0.04		B		
Pepo	Stored 10 Control	7.23	3	0.04		B		
Soler	Stored 10 MAP	6.99	3	0.04			C	
VL	Stored 10 MAP	6.85	3	0.04			C	D
Soler	Fresh	6.77	3	0.04				D
Pepo	Fresh	6.75	3	0.04				D
TD	Fresh	6.68	3	0.04				D
VL	Fresh	6.67	3	0.04				D

Means with the same letter are not significantly different with 0.05 probability level Tukey's test
TD = Taina Dorada
VL = Verde Luz
Pepo = Bush White Scallop

Table 16:Statistical analysis of the factorial design for the effect of pH on storage treatment for four genotypes of *Cucurbita* at 5°C

pH

Variable	N	R^2	R^2 Aj	CV
pH	27	0.97	0.96	0.66

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	1.30	10	0.13	60.40	<0.0001
Run	0.02	2	0.01	4.99	0.0207
Genotype	0.16	2	0.08	38.04	<0.0001
TRT	0.96	2	0.48	223.22	<0.0001
Genotype*TRT	0.15	4	0.04	17.87	<0.0001
Error	0.03	16	2.2E-03		
Total	1.33	26			

Test: Tukey Alfa=0.05 DMS=0.05644

Error: 0.0022 gl: 16

Run	Means	n	E.E.		
3	7.01	9	0.02	A	
2	6.99	9	0.02	A	B
1	6.94	9	0.02		B

Test: Tukey Alfa=0.05 DMS=0.13477

Error: 0.0022 gl: 16

Genotype	TRT	Means	n	E.E.				
TD	Stored 5 MAP	7.29	3	0.03	A			
TD	Stored 5 Control	7.27	3	0.03	A			
Soler	Stored 5 Control	7.09	3	0.03		B		
VL	Stored 5 Control	7.09	3	0.03		B		
VL	Stored 5 MAP	7.04	3	0.03		B		
Soler	Stored 5 MAP	6.90	3	0.03			C	
Soler	Fresh	6.77	3	0.03			C	D
TD	Fresh	6.71	3	0.03				D
VL	Fresh	6.67	3	0.03				D

Means with the same letter are not significantly different with 0.05 probability level Tukey's test
TD = Taina Dorada
VL = Verde Luz

Table 17: Statistical analysis of the factorial design for the effect of total acidity on storage treatment for four genotypes of *Cucurbita* at 10°*C*

Total acidity

Variable	N	R^2	R^2 Aj	CV
Total Acidity (%)	36	0.99	0.99	8.33

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-value
Model.	0.12	13	0.01	212.69	<0.0001
Run	5.4E-05	2	2.7E-05	0.64	0.5374
Genotype	2.0E-03	3	6.6E-04	15.68	<0.0001
TRT	0.11	2	0.06	1343.84	<0.0001
Genotype*TRT	1.2E-03	6	2.0E-04	4.84	0.0027
Error	9.3E-04	22	4.2E-05		
Total	0.12	35			

Test: Tukey Alfa=0.05 DMS=0.00667

Error: 0.0000 gl: 22

Run	Means	n	E.E.	
1	0.08	12	1.9E-03	A
2	0.08	12	1.9E-03	A
3	0.08	12	1.9E-03	A

Test: Tukey Alfa=0.05 DMS=0.01930

Error: 0.0000 gl: 22

Genotype	TRT	Means	n	E.E.				
Soler	Fresh	0.16	3	3.8E-03	A			
VL	Fresh	0.16	3	3.8E-03	A			
TD	Fresh	0.16	3	3.8E-03	A			
Pepo	Fresh	0.15	3	3.8E-03	A			
Pepo	Stored 10 MAP	0.06	3	3.8E-03		B		
Soler	Stored 10 MAP	0.06	3	3.8E-03		B	C	
Soler	Stored 10 Control	0.04	3	3.8E-03			C	D
VL	Stored 10 MAP	0.04	3	3.8E-03			C	D
Pepo	Stored 10 Control	0.04	3	3.8E-03			C	D
TD	Stored 10 MAP	0.03	3	3.8E-03				D
VL	Stored 10 Control	0.02	3	3.8E-03				D
TD	Stored 10 Control	0.02	3	3.8E-03				D

Means with the same letter are not significantly different with 0.05 probability level Tukey's test

TD = Taina Dorada

VL = Verde Luz

Pepo = Bush White Scallop

Table 18:Statistical analysis of the factorial design for the effect of total acidity on storage treatment for four genotypes of *Cucurbita* at 5°C

Total acidity

Variable	N	R^2	R^2 Aj	CV
Total Acidity (%)	27	0.9943	0.9908	7.9869

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	0.0978	10	0.0098	279.8819	<0.0001
Run	2.0E-05	2	9.9E-06	0.2825	0.7576
Genotype	0.0005	2	0.0003	7.7480	0.0044
TRT	0.0971	2	0.0486	1390.1557	<0.0001
Genotype*TRT	0.0001	4	2.1E-05	0.6116	0.6603
Error	0.0006	16	3.5E-05		
Total	0.0984	26			

Test: Tukey Alfa=0.05 DMS=0.00719

Error: 0.0000 gl: 16

Run	Means	n	E.E.		
3	0.0749	9	0.0020	A	
1	0.0743	9	0.0020	A	
2	0.0728	9	0.0020	A	

Test: Tukey Alfa=0.05 DMS=0.01717

Error: 0.0000 gl: 16

Genotype	TRT	Means	n	E.E.			
Soler	Fresh	0.1635	3	0.0034	A		
VL	Fresh	0.1573	3	0.0034	A		
TD	Fresh	0.1555	3	0.0034	A		
Soler	Stored 5 Control	0.0390	3	0.0034		B	
VL	Stored 5 Control	0.0355	3	0.0034		B	C
Soler	Stored 5 MAP	0.0355	3	0.0034		B	C
TD	Stored 5 Control	0.0302	3	0.0034		B	C
VL	Stored 5 MAP	0.0302	3	0.0034		B	C
TD	Stored 5 MAP	0.0194	3	0.0034			C

Means with the same letter are not significantly different with 0.05 probability level Tukey's test

TD = Taina Dorada

VL = Verde Luz

Table 19: Statistical analysis of the factorial design for the effect of ascorbic acid on storage treatment for four genotypes of *Cucurbita* at 10°C.

Ascorbic acid

Variable	N	R^2	R^2 Aj	CV
Vit. C content (mg/100g)	36	1.00	1.00	2.96

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	692.54	13	53.27	602.41	<0.0001
Run	0.01	2	0.01	0.06	0.9434
Genotype	0.77	3	0.26	2.90	0.0578
TRT	690.21	2	345.11	3902.45	<0.0001
Genotype*TRT	1.55	6	0.26	2.93	0.0298
Error	1.95	22	0.09		
Total	694.49	35			

Test: Tukey Alfa=0.05 DMS=0.30497

Error: 0.0884 gl: 22

Run	Means	n	E.E.	
3	10.08	12	0.09	A
1	10.05	12	0.09	A
2	10.04	12	0.09	A

Test: Tukey Alfa=0.05 DMS=0.38927

Error: 0.0884 gl: 22

Genotype	TRT	Means	n	E.E.				
VL	Fresh	16.15	3	0.17	A			
Pepo	Fresh	16.15	3	0.17	A			
Soler	Fresh	16.14	3	0.17	A			
TD	Fresh	16.14	3	0.17	A			
Pepo	Stored 10 MAP	8.07	3	0.17		B		
VL	Stored 10 MAP	8.06	3	0.17		B		
Soler	Stored 10 MAP	8.06	3	0.17		B		
TD	Stored 10 MAP	7.72	3	0.17		B		
Soler	Stored 10 Control	6.72	3	0.17			C	
TD	Stored 10 Control	6.03	3	0.17			C	D
VL	Stored 10 Control	5.76	3	0.17				D
Pepo	Stored 10 Control	5.66	3	0.17				D

Means with the same letter are not significantly different with 0.05 probability level Tukey's test
TD = Taina Dorada
VL = Verde Luz
Pepo = Bush White Scallop

Table 20:Statistical analysis of the factorial design for the effect of ascorbic acid on storage treatment for four genotypes of *Cucurbita* at 5°C.

Ascorbic acid

Variable	N	R^2	R^2 Aj	CV
Vit. C content (mg/100g)	27	0.93	0.89	21.70

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	805.14	10	80.51	22.89	<0.0001
Run	16.81	2	8.41	2.39	0.1236
Genotype	3.26	2	1.63	0.46	0.6373
TRT	782.84	2	391.42	111.26	<0.0001
Genotype*TRT	2.23	4	0.56	0.16	0.9563
Error	56.29	16	3.52		
Total	861.44	26			

Test: Tukey Alfa=0.05 DMS=2.28154

Error: 3.5182 gl: 16

Run	Means	n	E.E.	
3	9.42	9	0.63	A
2	8.95	9	0.63	A
1	7.56	9	0.63	A

Test: Tukey Alfa=0.05 DMS=5.44819

Error: 3.5182 gl: 16

Genotype	TRT	Means	n	E.E.		
VL	Fresh	16.15	3	1.08	A	
TD	Fresh	16.14	3	1.08	A	
Soler	Fresh	16.14	3	1.08	A	
Soler	Stored 5 Control	6.49	3	1.08		B
TD	Stored 5 Control	5.93	3	1.08		B
VL	Stored 5 Control	5.67	3	1.08		B
Soler	Stored 5 MAP	4.69	3	1.08		B
TD	Stored 5 MAP	3.58	3	1.08		B
VL	Stored 5 MAP	3.00	3	1.08		B

Means with the same letter are not significantly different with 0.05 probability level Tukey's test
TD = Taina Dorada
VL = Verde Luz

Table 21: Statistical analysis of the factorial design for the effect of beta-carotene on storage treatment for four genotypes of *Cucurbita* at 10°C

Beta-carotene

Variable	N	R^2	R^2 Aj	CV
B-carotene content (mg/100..	36	0.91	0.86	9.98

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	519.29	13	39.95	17.04	<0.0001
Run	18.51	2	9.26	3.95	0.0342
Genotype	205.35	3	68.45	29.20	<0.0001
TRT	206.44	2	103.22	44.04	<0.0001
Genotype*TRT	88.99	6	14.83	6.33	0.0006
Error	51.56	22	2.34		
Total	570.85	35			

Test: Tukey Alfa=0.05 DMS=1.57007

Error: 2.3439 gl: 22

Run	Means	n	E.E.		
3	16.26	12	0.44	A	
2	15.26	12	0.44	A	B
1	14.51	12	0.44		B

Test: Tukey Alfa=0.05 DMS=4.54708

Error: 2.3439 gl: 22

Genotype	TRT	Means	n	E.E.					
Pepo	Stored 10 Control	21.21	3	0.88	A				
Pepo	Stored 10 MAP	20.59	3	0.88	A				
TD	Stored 10 MAP	20.57	3	0.88	A				
VL	Stored 10 Control	18.17	3	0.88	A	B			
VL	Stored 10 MAP	15.22	3	0.88		B	C		
TD	Stored 10 Control	15.16	3	0.88		B	C	D	
Pepo	Fresh	14.12	3	0.88		B	C	D	E
Soler	Stored 10 MAP	13.00	3	0.88			C	D	E
VL	Fresh	12.75	3	0.88			C	D	E
Soler	Stored 10 Control	12.29	3	0.88			C	D	E
TD	Fresh	10.65	3	0.88				D	E
Soler	Fresh	10.38	3	0.88					E

Means with the same letter are not significantly different with 0.05 probability level Tukey's test
TD = Taina Dorada
VL = Verde Luz
Pepo = Bush White Scallop

Table 22:Statistical analysis of the factorial design for the effect of beta-carotene on storage treatment for four genotypes of *Cucurbita* at 5°C

Beta-carotene

Variable	N	R^2	R^2 Aj	CV
B-carotene (mg/100g)	27	0.87	0.79	7.15

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	90.23	10	9.02	10.52	<0.0001
Run	0.22	2	0.11	0.13	0.8809
Genotype	19.62	2	9.81	11.43	0.0008
TRT	59.76	2	29.88	34.83	<0.0001
Genotype*TRT	10.62	4	2.66	3.10	0.0458
Error	13.73	16	0.86		
Total	103.95	26			

Test: Tukey Alfa=0.05 DMS=1.12667

Error: 0.8579 gl: 16

Run	Means	n	E.E.	
1	13.05	9	0.31	A
3	12.96	9	0.31	A
2	12.83	9	0.31	A

Test: Tukey Alfa=0.05 DMS=2.69044

Error: 0.8579 gl: 16

Genotype	TRT	Means	n	E.E.				
TD	Stored 5 MAP	16.09	3	0.53	A			
VL	Stored 5 MAP	15.01	3	0.53	A	B		
TD	Stored 5 Control	13.92	3	0.53	A	B	C	
Soler	Stored 5 MAP	13.55	3	0.53	A	B	C	
VL	Stored 5 Control	12.87	3	0.53		B	C	D
VL	Fresh	12.75	3	0.53		B	C	D
Soler	Stored 5 Control	11.29	3	0.53			C	D
TD	Fresh	10.66	3	0.53				D
Soler	Fresh	10.38	3	0.53				D

Means with the same letter are not significantly different with 0.05 probability level Tukey's test
TD = Taina Dorada
VL = Verde Luz

Table 23:Statistical analysis of the factorial design for the effect of phenolic compounds on storage treatment for four genotypes of *Cucurbita* at 10°C

Total phenolics

Variable	N	R^2	R^2 Aj	CV
Total Phenolic (mg GAE/100g)	36	1.00	1.00	3.00

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	356564.91	13	27428.07	833.12	<0.0001
Run	151.41	2	75.71	2.30	0.1239
Genotype	1252.65	3	417.55	12.68	0.0001
TRT	354935.21	2	177467.60	5390.50	<0.0001
Genotype*TRT	225.64	6	37.61	1.14	0.3715
Error	724.29	22	32.92		
Total	357289.20	35			

Test: Tukey Alfa=0.05 DMS=5.88437

Error: 32.9223 gl: 22

Corrida	Means	n	E.E.	
2	192.97	12	1.66	A
3	191.92	12	1.66	A
1	188.19	12	1.66	A

Test: Tukey Alfa=0.05 DMS=17.04168

Error: 32.9223 gl: 22

Genotype	TRT	Means	n	E.E.				
Soler	Fresh	342.65	3	3.31	A			
VL	Fresh	332.47	3	3.31	A	B		
TD	Fresh	328.40	3	3.31	A	B		
Pepo	Fresh	322.29	3	3.31		B		
Soler	Stored 10 MAP	129.80	3	3.31			C	
Soler	Stored 10 Control	125.89	3	3.31			C	D
TD	Stored 10 MAP	125.69	3	3.31			C	D
VL	Stored 10 Control	122.59	3	3.31			C	D
TD	Stored 10 Control	119.90	3	3.31			C	D
VL	Stored 10 MAP	116.60	3	3.31			C	D
Pepo	Stored 10 Control	113.57	3	3.31			C	D
Pepo	Stored 10 MAP	112.49	3	3.31				D

Means with the same letter are not significantly different with 0.05 probability level Tukey's test
TD = Taina Dorada
VL = Verde Luz
Pepo = Bush White Scallop

Table 24:Statistical analysis of the factorial design for the effect of phenolic compounds on storage treatment for four genotypes of *Cucurbita* at 5°C

Total phenolics

Variable	N	R^2	R^2 Aj	CV
Total Phenolics (mg GAE/100g)	27	1.00	1.00	3.10

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	235945.17	10	23594.52	593.39	<0.0001
Run	64.90	2	32.45	0.82	0.4597
Genotype	678.64	2	339.32	8.53	0.0030
TRT	235168.06	2	117584.03	2957.17	<0.0001
Genotype*TRT	33.56	4	8.39	0.21	0.9285
Error	636.20	16	39.76		
Total	236581.36	26			

Test: Tukey Alfa=0.05 DMS=7.67018

Error: 39.7624 gl: 16

Corrida	Means	n	E.E.	
2	205.74	9	2.10	A
3	203.44	9	2.10	A
1	201.97	9	2.10	A

Test: Tukey Alfa=0.05 DMS=18.31600

Error: 39.7624 gl: 16

Genotype	TRT	Means	n	E.E.			
Soler	Fresh	342.65	3	3.64	A		
VL	Fresh	332.47	3	3.64	A		
TD	Fresh	328.40	3	3.64	A		
Soler	Stored 5 Control	158.62	3	3.64		B	
VL	Stored 5 Control	152.15	3	3.64		B	
TD	Stored 5 Control	150.27	3	3.64		B	
Soler	Stored 5 MAP	130.72	3	3.64			C
VL	Stored 5 MAP	119.80	3	3.64			C
TD	Stored 5 MAP	118.38	3	3.64			C

Means with the same letter are not significantly different with 0.05 probability level Tukey's test
TD = Taina Dorada
VL = Verde Luz

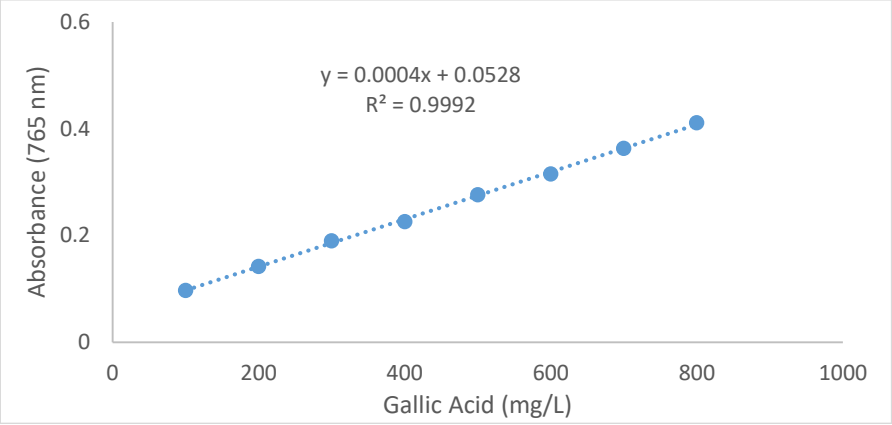


Figure 46: Phenolic compound standard curve using Gallic acid equivalent (GAE)

Table 25: Statistical analysis of the factorial design for the effect of antioxidant activity on storage treatment for four genotypes of *Cucurbita* at 10°C

DPPH

Variable	N	R^2	R^2 Aj	CV
DPPH content (uM TEAC/g)	36	0.99	0.99	8.46

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	32271.27	13	2482.41	306.10	<0.0001
Run	13.09	2	6.55	0.81	0.4589
Genotype	4287.83	3	1429.28	176.24	<0.0001
TRT	23847.88	2	11923.94	1470.30	<0.0001
Genotype*TRT	4122.46	6	687.08	84.72	<0.0001
Error	178.42	22	8.11		
Total	32449.68	35			

Test: Tukey Alfa=0.05 DMS=2.92053

Error: 8.1099 gl: 22

Run	Means	n	E.E.	
3	34.37	12	0.82	A
2	33.68	12	0.82	A
1	32.89	12	0.82	A

Test: Tukey Alfa=0.05 DMS=8.45813

Error: 8.1099 gl: 22

Genotype	TRT	Means	n	E.E.					
VL	Fresh	94.21	3	1.64	A				
Soler	Fresh	91.83	3	1.64	A				
TD	Fresh	53.93	3	1.64		B			
Pepo	Fresh	38.78	3	1.64			C		
VL	Stored 10 MAP	35.62	3	1.64			C		
TD	Stored 10 MAP	24.77	3	1.64				D	
Soler	Stored 10 Control	14.09	3	1.64					E
VL	Stored 10 Control	13.26	3	1.64					E
TD	Stored 10 Control	11.08	3	1.64					E
Soler	Stored 10 MAP	10.39	3	1.64					E
Pepo	Stored 10 MAP	9.29	3	1.64					E
Pepo	Stored 10 Control	6.49	3	1.64					E

Means with the same letter are not significantly different with 0.05 probability level Tukey's test
TD = Taina Dorada
VL = Verde Luz
Pepo = Bush White Scallop

Table 26: Statistical analysis of the factorial design for the effect of antioxidant activity on storage treatment for four genotypes of *Cucurbita* at 5°C

DPPH

Variable	N	R^2	R^2 Aj	CV
DPPH content (uM TEAC/g)	27	0.99	0.99	4.26

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	14075.01	10	1407.50	255.25	<0.0001
Run	0.07	2	0.04	0.01	0.9935
Genotype	3015.05	2	1507.53	273.39	<0.0001
TRT	9597.05	2	4798.53	870.20	<0.0001
Genotype*TRT	1462.83	4	365.71	66.32	<0.0001
Error	88.23	16	5.51		
Total	14163.24	26			

Test: Tukey Alfa=0.05 DMS=2.85636

Error: 5.5142 gl: 16

Run	Means	n	E.E.	
2	55.13	9	0.78	A
3	55.04	9	0.78	A
1	55.01	9	0.78	A

Test: Tukey Alfa=0.05 DMS=6.82083

Error: 5.5142 gl: 16

Genotype	TRT	Means	n	E.E.			
VL	Fresh	94.21	3	1.36	A		
Soler	Fresh	91.83	3	1.36	A		
Soler	Stored 5 Control	60.23	3	1.36		B	
VL	Stored 5 Control	58.99	3	1.36		B	
TD	Fresh	53.93	3	1.36		B	
Soler	Stored 5 MAP	35.46	3	1.36			C
VL	Stored 5 MAP	34.47	3	1.36			C
TD	Stored 5 MAP	33.30	3	1.36			C
TD	Stored 5 Control	33.11	3	1.36			C

Means with the same letter are not significantly different with 0.05 probability level Tukey's test

TD = Taina Dorada

VL = Verde Luz

Pepo = Bush White Scallop

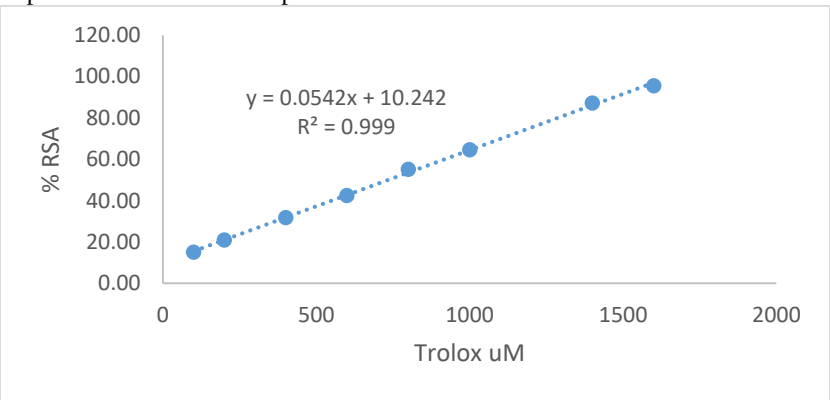


Figure 47:Antioxidant activity standard curve using Trolox equivalent (TEAC)

8.6. Proximal Analysis and micronutrients of *Cucurbita* flowers

Moisture

Variable	N	R²	R² Aj	CV
Moisture Content	48	0.12	0.06	0.65

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	2.32	3	0.77	2.09	0.1154
Genotype	2.32	3	0.77	2.09	0.1154
Error	16.27	44	0.37		
Total	18.58	47			

Test: Tukey Alfa=0.05 DMS=0.66278

Error: 0.3697 gl: 44

Genotype	Means	n	E.E.		
Verde Luz	94.25	12	0.18	A	
Taina Dorada	94.12	12	0.18	A	
Pepo	93.79	12	0.18	A	
Soler	93.72	12	0.18	A	

Ash

Variable	N	R²	R² Aj	CV
Ash content	51	0.17	0.12	10.41

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Modelo.	0.05	3	0.02	3.19	0.0319
Genotype	0.05	3	0.02	3.19	0.0319
Error	0.27	47	0.01		
Total	0.32	50			

Test: Tukey Alfa=0.05 DMS=0.07982

Error: 0.0057 gl: 47

Genotype	Means	n	E.E.			
Soler	0.78	12	0.02	A		
Verde Luz	0.73	14	0.02	A		B
Pepo	0.70	12	0.02			B
Taina Dorada	0.70	13	0.02			B

Protein

Variable	N	R²	R² Aj	CV
Protein	12	0.95	0.93	3.63

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Modelo.	0.52	3	0.17	53.17	<0.0001
Genotype	0.52	3	0.17	53.17	<0.0001
Error	0.03	8	3.3E-03		
Total	0.55	11			

Test: Tukey Alfa=0.05 DMS=0.14962

Error: 0.0033 gl: 8

Genotype	Means	n	E.E.			
Soler	1.91	3	0.03	A		
Pepo	1.60	3	0.03		B	
Taina Dorada	1.43	3	0.03			C
Verde Luz	1.37	3	0.03			C

Fat

Variable	N	R²	R² Aj	CV
Fat content	34	0.65	0.62	17.56

Analysis table of variance (SC type III)

F.V.	SC	gl	CM	F	p-value
Model.	0.02	3	0.01	18.82	<0.0001
Genotype	0.02	3	0.01	18.82	<0.0001
Error	0.01	30	4.0E-04		
Total	0.03	33			

Test: Tukey Alfa=0.05 DMS=0.02634

Error: 0.0004 gl: 30

Genotype	Means	n	E.E.		
Taina Dorad	0.15	8	0.01	A	
Verde Luz	0.13	8	0.01	A	
Soler	0.09	9	0.01		B
Pepo	0.09	9	0.01		B

Mineral Measures

Mineral	Genotype	Variable	n	Means	E.E.
Ca	Pepo	mg/100g	3	45.07	6.71
Ca	Soler	mg/100g	3	58.40	6.66
Ca	Taina Dorada	mg/100g	3	52.22	2.60
Ca	Verde Luz	mg/100g	3	53.80	2.82
Fe	Pepo	mg/100g	3	1.07	0.01
Fe	Soler	mg/100g	3	2.07	0.06
Fe	Taina Dorada	mg/100g	3	1.06	0.13
Fe	Verde Luz	mg/100g	3	1.20	0.01
K	Pepo	mg/100g	3	278.59	0.90
K	Soler	mg/100g	3	290.76	5.34
K	Taina Dorada	mg/100g	3	231.87	2.41
K	Verde Luz	mg/100g	3	278.11	1.33
Mg	Pepo	mg/100g	3	24.18	0.62
Mg	Soler	mg/100g	3	25.96	1.27
Mg	Taina Dorada	mg/100g	3	23.76	1.53
Mg	Verde Luz	mg/100g	3	20.50	1.42
Mn	Pepo	mg/100g	3	0.24	2.0E-03
Mn	Soler	mg/100g	3	0.17	0.01
Mn	Taina Dorada	mg/100g	3	0.21	0.01
Mn	Verde Luz	mg/100g	3	0.13	3.5E-03
P	Pepo	mg/100g	3	39.47	0.55
P	Soler	mg/100g	3	33.91	1.31
P	Taina Dorada	mg/100g	3	31.02	1.61
P	Verde Luz	mg/100g	3	31.26	0.81
Zn	Pepo	mg/100g	3	0.11	0.01
Zn	Soler	mg/100g	3	0.17	0.01
Zn	Taina Dorada	mg/100g	3	0.16	0.01
Zn	Verde Luz	mg/100g	3	0.13	0.01

8.7. Sensory Evaluations

Universidad de Puerto Rico, Recinto Universitario de Mayagüez
Programa de Ciencias y Tecnología de Alimentos
Prueba Sensorial

Fecha: _____ No. panelista: _____

Producto: **Flor de Calabaza Fresca**

Instrucciones:
Se le está entregando una muestra de flor de calabaza fresca. Anote el número de la muestra en el espacio provisto abajo. Luego, pruebe la muestra y tome agua entre cada degustación con el fin de limpiar los receptores. Finalmente, determine cuanto le gusta o disgusta la muestra utilizando la escala de 1 al 9. Si tiene algún comentario lo puede añadir en el espacio provisto al final de la hoja.

Número de Muestra: _____

Aceptación General

9. Me gusta extremadamente	☐
8. Me gusta mucho	☐
7. Me gusta moderadamente	☐
6. Me gusta ligeramente	☐
5. Ni me gusta ni me disgusta	☐
4. Me disgusta ligeramente	☐
3. Me disgusta moderadamente	☐
2. Me disgusta mucho	☐
1. Me disgusta extremadamente	☐

¿Usted compraría este producto? _____

Comentarios:
_____
_____
_____
_____
_____

Figure 48: Panelist sheet for evaluating overall acceptability on fresh organic pumpkin flowers.

Universidad de Puerto Rico, Recinto Universitario de Mayagüez
Programa de Ciencias y Tecnología de Alimentos
Prueba Sensorial

Fecha: _____ No. panelista: _____

Producto: **Flor de Calabaza Fresca**

Instrucciones:

Se le está entregando una muestra de flor de calabaza fresca. Anote el número de la muestra en el espacio provisto abajo. Luego, pruebe la muestra y tome agua entre cada degustación con el fin de limpiar los receptores. Finalmente, determine cuanto le gusta o disgusta el sabor de la muestra utilizando la escala de 1 al 9. Si tiene algún comentario lo puede añadir en el espacio provisto al final de la hoja.

Número de Muestra: _____

	Sabor
9. Me gusta extremadamente	☐
8. Me gusta mucho	☐
7. Me gusta moderadamente	☐
6. Me gusta ligeramente	☐
5. Ni me gusta ni me disgusta	☐
4. Me disgusta ligeramente	☐
3. Me disgusta moderadamente	☐
2. Me disgusta mucho	☐
1. Me disgusta extremadamente	☐

Comentarios:

_____
_____
_____
_____
_____

Figure 49: Panelist sheet for evaluating overall taste on fresh organic pumpkin flowers

Universidad de Puerto Rico, Recinto Universitario de Mayagüez
Programa de Ciencias y Tecnología de Alimentos
Prueba Sensorial

Fecha: _____ No. Panelista: _____

Producto: **Flor de Calabaza Fresca**

Definición de textura: La palabra textura está relacionada con el frescor crujiente, tierno y suculento. Que son términos que se aplican en grados variables para definir la calidad de las hortalizas de hoja en este caso seria los pétalos de la flor de calabaza fresca.

Instrucciones:
Se le está entregando una muestra de flor de calabaza fresca. Anote el número de la muestra en el espacio provisto abajo. Luego, pruebe la muestra y tome agua entre cada degustación con el fin de limpiar los receptores. Finalmente, determine cuanto le gusta o disgusta la textura de la muestra utilizando la escala de 1 al 9. Si tiene algún comentario lo puede añadir en el espacio provisto al final de la hoja.

Número de Muestra: _____

	Textura
9. Me gusta extremadamente	☐
8. Me gusta mucho	☐
7. Me gusta moderadamente	☐
6. Me gusta ligeramente	☐
5. Ni me gusta ni me disgusta	☐
4. Me disgusta ligeramente	☐
3. Me disgusta moderadamente	☐
2. Me disgusta mucho	☐
1. Me disgusta extremadamente	☐

Comentarios:

_____
_____
_____
_____
_____
_____

Figure 50: Panelist sheet for evaluating overall texture on fresh organic pumpkin flowers

Universidad de Puerto Rico, Recinto Universitario de Mayagüez
Programa de Ciencias y Tecnología de Alimentos
Prueba Sensorial

Fecha: _____

Producto: Flor de Calabaza Cocida
No. panelista: _____
Instrucciones:
Se le está entregando una muestra de flor de calabaza cocida. Anote el número de la muestra en el espacio provisto abajo. Luego, pruebe la muestra y tome agua entre cada degustación con el fin de limpiar los receptores. Finalmente, determine cuanto le gusta o disgusta la muestra utilizando la escala de 1 al 9. Si tiene algún comentario lo puede añadir en el espacio provisto al final de la hoja.

Número de Muestra: _____

	Sabor General
9. Me gusta extremadamente	☐
8. Me gusta mucho	☐
7. Me gusta moderadamente	☐
6. Me gusta ligeramente	☐
5. Ni me gusta ni me disgusta	☐
4. Me disgusta ligeramente	☐
3. Me disgusta moderadamente	☐
2. Me disgusta mucho	☐
1. Me disgusta extremadamente	☐

Comentarios:
_____
_____
_____
_____
_____

Figure 51: Panelist sheet for evaluating overall taste for minimally processed pumpkin flowers