Investigation note

Post-harvest quality of squash fruits stored at low temperature

Laura Angélica Montecinos-Pedro[1]
Ma. de Lourdes Arévalo-Galarza[1§]
Cecilia García-Osorio[1]
Jorge Cadena-Iñiguez[2]
Martha Elva Ramírez-Guzmán[3]

[1]Montecillo *Campus*-Postgraduate College. Mexico-Texcoco Highway km 36.5, Montecillo, Texcoco, State of Mexico. CP. 56230. (montecinos.laura@colpos.mx; gcecilia@colpos.mx). [2]San Luis Potosí *Campus*-Postgraduate College. Salinas de Hidalgo, San Luis Potosí, Mexico. CP. 78622. (jocadena@colpos.mx). [3]Postgraduate in Statistics-Postgraduate College. Mexico-Texcoco Highway km 36.5, Montecillo, Texcoco, State of Mexico. CP. 56230. (martarg@colpos.mx).

[§]Corresponding author: larevalo@colpos.mx.

Abstract

The squash *nigrum spinosum* is one of the main genotypes that are commercialized in Mexico, the United States of America and Canada, with the Latin and Asian communities being the main consumers. Generally, the works reported in this species are focused on the genus *virens levis*, but there is very little information on the post-harvest behavior of genotype *n. spinosum* under refrigerated storage. For this, fruits were harvested in horticultural maturity and stored at room temperature (20 ±2 °C) and at low temperature (10 ±1 °C, 85% RH) for three periods of time (3, 6 and 9 weeks) with and without the application of 1-methylcyclopropene (0, 500 and 1 000 nL L^{-1}) to reduce viviparism. The variables evaluated were humidity percentage, titratable acidity, SST (ºBrix), chlorophyll *a* and *b* content and total, content of total sugars (sucrose, fructose and glucose), weight loss and percentage of viviparism. The results showed an average moisture content of 94%, with low SST content (4.5-5.2%) that remains unchanged during storage, a fructose and glucose content of 1.07% and 0.89% respectively, without the presence of sucrose. The fruits maintained their appearance characteristics with refrigerated storage for 3 weeks, but at 6 and 9 weeks, the dehydration of thorns was evident, although both doses of 1-MCP (500 and 1000 nL L^{-1}) significantly reduced the viviparism, increased the susceptibility to the incidence of fungi mainly *Fusarium* sp.

Keywords: chlorophyll, dehydration, thorns, viviparism.

Reception date: July 2019
Acceptance date: August 2019

The squash (*Sechium edule* Jacq. Sw.) whose name in Nahuatl chayotl means 'spiny gourd', is native to Mesoamerica and has a wide variation in the shape and color of fruits (SINAREFI, 2015). This fruit has diuretic and anti-inflammatory properties, prevents renal calcification and arteriosclerosis, which reduces cardiovascular risks (Jensen and Lai, 1986). Of the broad biological richness of the squash, two genotypes are commercialized in Mexico: *virens levis* (smooth green) and *nigrum spinosum* (black spiny). Both genotypes are exported to the United States of America and Canada due to the growing Latin and Asian population.

The shelf life of the fruits is limited by the loss of color, dehydration and presence of viviparism (germination of the seed inside the fruit), which is one of the factors that limits the shelf life and is a reason for rejection during commercialization in both fruit genotypes. Viviparism is a phenomenon associated with ethylene biosynthesis, so that this growth regulator acts must be recognized by the receptor, leading to the transcriptional activation of genes that trigger various physiological responses such as germination (Sisler and Serek, 1997; Blankenship, 2001).

Recent studies show that the application of 1-MCP (1-methylcyclopropene) prevents the recognition of the receptor to ethylene, since it has greater affinity, and cannot activate the mechanisms that lead to the processes of germination so that some effectiveness in delaying the Viviparism. In the case of the squash *virens levis*, when 300 nL of 1-MCP was applied to fruits stored for 28 days at 10 °C, plus 6 at room temperature, only 5% of the total fruits were observed to be viviparous compared to 50% of the control fruits (Cadena-Iñiguez *et al*., 2006). However, it is unknown if 1-MCP can have the same effectiveness in the fruits of the genotype *nigrum spinosum*, since there is no information on this.

Therefore, the objective of this study was to evaluate and generate information on the effects of the use of 1-MCP on shelf life and post-harvest quality under refrigerated storage of squash *nigrum spinosum*.

Vegetal material

120 fruits were harvested, healthy, without wounds, in horticultural maturity and without presence of viviparism (Figure 1).

Figure 1. a) harvest and b): appearance of squash *nigrum spinosum* fruits from the Germplasm Bank of *Sechium edule* (BANGESe) in Huatusco Veracruz.

The harvest was carried out in November 2016, in the National Germplasm Bank of *Sechium edule* (BANGESe), located in the Regional University Center Orient (CRUO), which belongs to the Autonomous University Chapingo (UACH) in the municipality of Huatusco, Veracruz, Mexico (19° 08' 48'' north latitude and 97° 57' 00" west latitude).

The vegetation of the place is mesophilic forest of mountain (1 340 m of altitude) and average annual temperature of 19 °C and 85% RH, with 2 250 mm of annual average precipitation (Cadena-Iñiguez *et al*., 2006). The fruits were transported in plastic boxes to the Postharvest laboratory of the College of Postgraduates, Montecillo, Mexico.

The squashs were immersed in a solution of sodium hypochlorite (0.1%) for 2 min, then allowed to dry at room temperature for 3 h. The fruits were divided into 12 batches of 10 fruits each, considering each batch one treatment (4 storage times: 0 (room temperature) 3, 6, 9 weeks (refrigeration) and 3 doses of 1-MCP (0, 500 and 100 nL L^{-1}) The application of 1-MCP (SmartFresh®, 14%, Rohm and Haas Co.) was by exposure for 6 h at 20 °C, placing the fruits in a hermetically sealed package where a vial was placed the determined concentration of 1-MCP. The fruits were then kept at room temperature (20 ±2 °C) and under refrigeration at 10 ±1 °C for different periods of time according to the treatment.

The variables weight loss and viviparism were measured every two days without destroying the fruits, both at room temperature and at the exit from the cold room. While the chemical analyzes were carried out at the exit of the cold room and on day 11.

Variables evaluated

Humidity percentage

A slice of 1 cm thick was taken from the center of the squash, (without epidermis or seed) and placed inside a mechanical convection oven (Lab-Line Imperial V, Alpha Multiservises, Inc. USA) at 50 °C for 8 days obtaining a constant weight. Moisture was calculated with the equation:

$$\text{Humidity percentage (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} * 100$$

Titratable acidity

It was determined by the volumetric method of the AOAC (1990) in 2 g of liquified pulp in 10 mL of distilled water. An aliquot of 5 mL was taken and 3 drops of phenolphthalein indicator were added. Subsequently, it was titrated with NaOH until the vire. The result was expressed as a percentage of citric acid.

Determination of SST (ºBrix)

It was determined with a digital refractometer (PAL-1, Atago™, Japan) in 5 fruits in each evaluation period, for which cuts were made in two areas of the equatorial region of each fruit at a depth of ±2.5 cm (mesocarp) and subsequently squeezed with a cloth.

Chlorophyll content

2 g of the pulp were placed in a vial with a lid, 10 mL of acetone (80%) were added and stored under dark conditions for 24 h and at room temperature. Then, the extract was measured at three wavelengths: 470, 646 and 663 nm in a UV spectrophotometer (Thermo Scientific™, modelo Genesys™ 10UV). To obtain the concentrations of chlorophylls (mg g^{-1}) the equations for the 80% acetone solvent (v/v) were applied, according to Lichtenthaler (1987):

Chlorophyll a: $C_a = (12.25*A_{663} - 2.79*A_{646})$

Chlorophyll b: $C_b = (21.5*A_{646} - 5.1*A_{663})$

Total chlorophyll: $C_{a+b} = (7.15*A_{663} - 18.71*A_{646})$

Total carotenoids: $C_{x+c} = \frac{1000*A_{470} - 1.82C_a - 85.02C_b}{198}$

Total sugar content by HPLC

For the mother sample, 5 g of finely chopped squash pulp was placed in a flask and 60 mL of 80% ethanol was added, covering it with a piece of aluminum foil. It was left to stand for 24 h at room temperature and subsequently it was concentrated. The solution was filtered with an acrodisk (Titan, 0.45 µm) and placed in a vial and analyzed by HPLC (High-Performance Liquid Chromatography) (series 200, Perkin Elmer™) with autosampler and refractive index (IR) detector. A Pinnacle II amino column of 5 mm 150 x 4.6 mm (Restek™) was used, the mobile phase was an acetonitrile/water solution (80:20) (v/v) with a running time of 14 min.

For the calibration curves, 0.05 g of fructose, glucose and 99.5% sucrose (Sigma-Aldrich, USA) in 10 mL of methanol: water (1:9) (v/v) were weighed separately, the corresponding dilutions were made (0.15 to 5 mg mL^{-1}). The conditions of the chromatograph were 35 °C, flow of 1 mL min^{-1}, with an injection volume of 10 µL.

Weight loss

The weight of each squash that remained at room temperature was recorded every two days until viviparism was found, calculating it with the following equation:

$$(\%)\ \text{weight loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} * 100$$

Viviparism

Every two days the presence of viviparism was recorded according to the opening level of the basal part of the fruit (Figure 2), being: level 0= absence of seed (nominal value 0), level 1: basal opening of the fruit with the visible seed, (nominal value 2), level 2: exposed seed of the fruit, (nominal value 4), with the following formula: % viviparism= ((n x v)/tf)x100; where n= number of fruits, v= level of viviparism; tf= total fruits under evaluation (Figure 2).

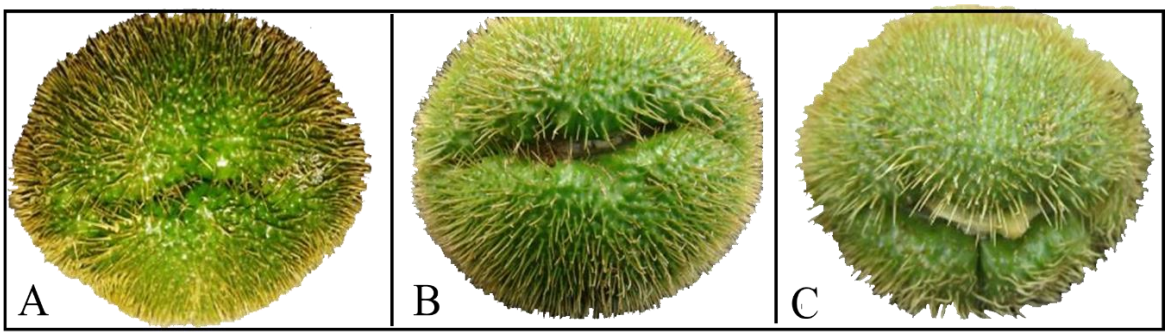

Figure 2. Classification of viviparism in squash *nigrum spinosum*. A: level 0= 0; B: level 1= 2; C: level 2= 4.

Experimental design

For the quality variables (% SST, chlorophyll content, titratable acidity and humidity percentage), a model with a 4 x 3 x 2 factorial experimental design was used. Factor levels were: week (0, 3, 6, 9), 1-MCP dose (0, 500, 1 000) and evaluation days (1 and 11).

$$Y_{ijk}=\mu + S_i + M_j + D_k + SM_{ij} + SD_{ik} + MD_{jk} + SMD_{ijk} + \varepsilon_{ijk}$$

Where: Y_{ijk}= response variable from the i-th week in the j-th dose of 1- MCP on the kth day; μ= average general; S_i= effect of the ith week (i = 0, 3, 6.9); M_j= effect of the j-th dose of 1-MCP (j = 0, 500, 1000); D_k= effect of the i-th day (k= 1, 11); SM_{ijk}= week interaction∗dose of 1- MCP; SMDijk= week interaction∗dose of 1- MCP∗day; ε_{ijk}= experimental error; where: N (0, σ2).

In the case of the variables: content of total sugars, fructose, glucose by HPLC, a 4 x 3 factorial experimental design was carried out. The factors were: storage weeks and 1-MCP dose. The levels of the factors were: week (0, 3, 6, 9), dose of 1-MCP (0, 500, 1 000). For the relationship between the variables of weight loss (%) and viviparism (%), a correlation analysis was performed to obtain the Pearson correlation coefficient (α= 0.05). All the data were analyzed with the statistical software InfoStat (Di-Rienzo *et al*., 2016).

Discussion

An excellent quality of the fruits of spiny squash is appreciated by the dark green color of the exocarp, uniform size, firm spines and absence of viviparism. These fruits are distinguished from the *virens levis* because they are juicier and in their composition are phenolic compounds (525 mg 100 g^{-1}) and cucurbitacins (137 mg 100 g^{-1}). According to Table 1, the fruits of *nigrum spinosum* have an average dry matter content between 5.1 and 6.1%, showing a lower humidity in the fruits maintained at room temperature and in those stored 9 weeks and with the highest dose of 1-MCP. Because the fruits of squash are harvested in horticultural maturity, there were no significant changes in their composition or storage effect, with values of total acidity and total soluble solids low (0.1% and between 4 and 5 °Bx, respectively) (Table 1). These values are similar to those reported in cucumber (*Cucumis sativus*), a vegetable belonging to the Cucurbit family, whose values are between 3 and 4 °Bx (Barraza-Álvarez, 2015).

It can be observed that the chlorophyll *a* and *b* contents of the fruits *nigrum spinosum* ranges from 3.94 to 7.44 mg 100 g^{-1}, with a higher concentration of chlorophyll *b* than chlorophyll *a*, which explains its green color, as well as its origin from temperate zones and valleys high. The cultivation of spiny squash predominates in altitudes superior to the mesophilic forest (1 600 to 2 800 masl) in the States of Veracruz, Michoacan, Puebla and Mexico, under these conditions of height and environment, generally the concentration of chlorophyll is greater in order to take better advantage of the scarce incident radiation. On the other hand, in the case of the fruits of the genus *virens levis*, cultivated in places of lower altitude, their color is green-yellow and with a significantly lower concentration of chlorophyll than *nigrum spinosum* (Azcon-Bieto and Talon, 2003; Cadena *et al*., 2007) (Table 1).

Table 1. Humidity content, titratable acidity (%AT), total soluble solids (%SST) and chlorophyll (mg 100 g^{-1}) in squash *nigrum spinosum* under different storage times and 1-MCP dose.

Factor		Level	Humidity (%)	AT (%)	SST (°Bx)	C_a (mg100g^{-1})	C_b (mg100 g^{-1})
SEAL	TA	0	93.89 b	0.11 ab	4.46 b	1.47 c	2.06 c
	R	3	94.52 a	0.09 c	4.99 a	2.47 bc	3.7 bc
		6	94.56 a	0.12 a	5.17 a	4.75 a	6.97 a
		9	94.13 b	0.1 bc	5.14 a	3.14 b	4.58 b
Dose 1-MCP		0	94.9 a	0.09 b	5.15 a	2.27 b	3.41 a
		500	93.69 c	0.11a	4.76 b	3.11 ab	4.59 a
		1 000	94.23 b	0.11a	4.9 b	3.49 a	4.98 a
DSA		1	94.24 a	0.11	4.99 a	2.78 a	4.14 a
		11	94.31 a	0.1	4.89 a	3.13 a	4.52 a
Week∗1-MCP			*	*	*	*	*
Week∗day			*	*	*	*	*
1-MCP∗day			*	*	ns	ns	ns
Week∗1-MCP∗day			*	*	*	ns	ns

SEAL= weeks of storage; AT= titratable acidity; TA= ambient temperature at 20 ±2 °C; R= cooling at 10 °C; DSA= days of sampling during simulation on shelf; 1-MCP= dose of 1-MCP; SST= total soluble solids; C_a= chlorophyll a; C_b= chlorophyll b; C_{a+b}= total chlorophyll; equal letters for each factor with their levels are not significantly different; ns= not significant; *= significant at $p \leq 0.05$.

Regarding the content of sugars, it varied from 1.23 to 3.45% and an average of 2.03%, the fructose content being slightly higher than glucose 1.07 and 0.89% respectively and without the presence of sucrose, without significant differences due to the effect of storage time, or 1-MCP application. The content of sugars in fruits of cucurbitaceas is diverse, in case of fruits of melon (*Cucumis melo* L.) genotype *cantalupensis* the content of total sugars is reported between 10.2% with sucrose as main sugar (4.35%) followed by fructose 1.95% and glucose 2.1% (Stepansky *et al*., 1999).

In the case of the zucchini (*Cucurbita maxima* var. Zapallito (Carr.) Millan) a lower total sugar content is reported with 4.27% (Massolo, 2013). The above shows that the squash has a very low content of sugars, compared to fruits of the same family, so it is recommended in hospital diets not only for its low caloric content but for the high content of dietary fiber superior to fruits such as plum, kiwi, or mango (Cadena-Iñiguez *et al*., 2007; Morillas-Ruiz and Delgado-Alarcón, 2012).

Regarding weight loss, it was observed that the fruits maintained at room temperature were similar to the fruits treated with 500 nL L^{-1} and the incidence of viviparism of 30 and 10%, respectively, on day 11 of storage, while the fruits treated with 1 000 nL L^{-1} had only 10% of viviparism on the same day.

For refrigerated storage, after 3 weeks the control fruits showed greater weight losses, with 60% viviparism, while the fruits treated with both doses of 1-MCP did not present this problem during the 11 d of storage, which indicates that together with the low temperature the application of 1-MCP inhibited the viviparism acting synergistically (Figure 3).

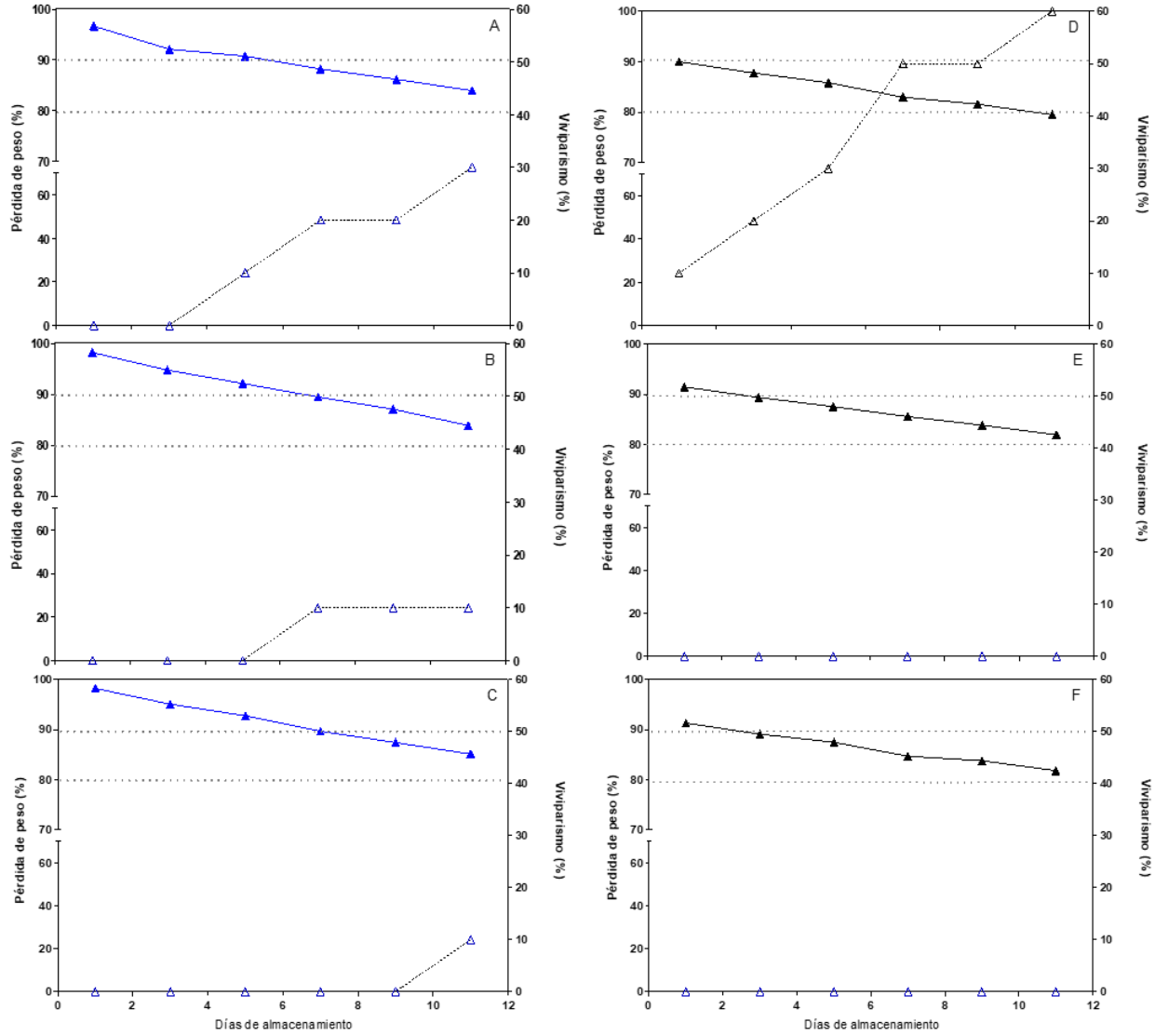

Figure 3. Percentage of weight loss and viviparism of squash *virens levis* fruits. Graphs A: (0 nL L^{-1}, 1-MCP), B: (500 nL L^{-1}, 1-MCP) and C: (1 000 nL L^{-1}, 1-MCP) maintained at room temperature and D (0 nL L^{-1}, 1-MCP), E (500 nL L^{-1}, 1-MCP) and F (1 000 nL L^{-1}, 1-MCP) fruits stored 3 weeks at 10 °C and kept at room temperature for 11 days.

Although 1-MCP is used mainly to delay the ripening process of climacteric fruits and in ornamental plants successfully, it has been used in a limited way in non-climacteric products, presenting diverse responses. For example, in strawberry, the application of 1-MCP between 1-1 000 nL L^{-1} maintained the color and the firmness (Jiang *et al*., 2001), in cucumber little or no beneficial effect was observed by the application of 1 MCP unless there is a risk that ethylene is present (Nilsson, 2005). In general, a positive correlation was observed between the loss of weight and viviparism of 0.819, while there was no significant correlation between the doses of 1-MCP with viviparism and weight loss.

As shown in Figure 4, the limit storage time to maintain the quality of fruits of *nigrum spinosum* in this work was 3 weeks at 10 °C, since for the 6 and 9 weeks of storage the spines of the fruits were dehydrated and presence of a high incidence of fungi, which was accentuated with the fruits treated with 1-MCP (Figure 4).

Figure 4. Appearance of squash *nigrum spinosum* fruits stored at different times (room temperature, 3s (three weeks), 6s (six weeks) and 9s (nine weeks) with the application of 1-MCP (0, 500 and 1 000 nL L-1) Initial (day 0), storage output (day 1) and 11 days after storage (day 11).

Conclusions

The fruits of squash *nigrum spinosum* did not present significant change in their composition when stored at room temperature (20 ±2 °C) and at low temperature (10 ±1 °C, 85% RH), they also contain lower humidity (5.1 to 6.1%) that the genus *virens levis*, also preserved their appearance characteristics with storage for 3 weeks, but not at 6 and 9 weeks since the fruit and spine presented dehydration and an opaque green color, the doses of 1-MCP (500 and 1 000 nL L^{-1}) significantly reduced the viviparism but increased the susceptibility to the incidence of fungi, developing rot in the fruit.

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