Physicochemical analysis in *Averrhoa carambola* L., var. Golden star and Arkin, in two post-harvest periods

Análisis físico-químico en *Averrhoa carambola* L., var. Golden star y Arkin, en dos estadios post-cosecha

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**Abstract**

Golden Star (GS) and Arkin (Ar) are important varieties of carambola fruits cultivated in México. Fruits were collected in a plantation of Jalisco and their physicochemical characterization was performed either in fresh or lyophilized fruits in two post-harvest time-points: immediately after harvest (IH-0) and 10 days post-harvest (PH-10), at room temperature. In IH-0, in GS the content of starch, glucose and total reducing sugars (TRS) was higher, while fructose was reduced. At PH-10, the size of GS fruits decreased, whereas an increase in total soluble solids and acidity in Ar fruits contrasted with a reduced pH and TRS content. Non-structural carbohydrates (NSCs) increased, from IH-0 to PH-10 in both varieties. Pectinolytic activity was highest in GS and Ar at PH-10, as was amylolytic activity. However, both activities were higher in Ar. Lyophilization significantly decreased the protein and starch contents, particularly in PH-10 fruits, whereas NSCs increased considerably. These results indicated a contrasting post-harvest behavior between the two varieties. The reported findings could be used to improve post-harvest management of carambola fruits.

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**Resumen**

Golden Star (GS) y Arkin (Ar) son variedades de carambola importantes en México. Se colectaron frutos en una plantación en Jalisco, México, y se caracterizaron fisicoquímicamente, tanto en fresco y liofilizado como en dos estados post-cosecha: corte inmediato (CI-0) y después del corte (DC-10) a temperatura ambiente. En CI-0, en GS el peso, el contenido de almidón, glucosa y azúcares reductores totales (ATR) fue mayor; pero menor de fructosa. En DC-10, GS disminuyó el tamaño y Ar incrementó en sólidos solubles totales y acidez, con una disminución del pH y ATR. El contenido de carbohidratos no estructurales (CNE) aumentó, de CI-0 a DC-10 en ambas variedades. La actividad pectinolítica fue más alta en GS y Ar a DC-10, al igual que la actividad amiloíntica. No obstante, ambas actividades fueron mayores en Ar. La liofilización disminuyó el contenido de proteína y almidón, principalmente en DC-10, mientras que los CNE aumentaron considerablemente. Estos resultados indicaron un comportamiento post-cosecha contrastante en estas variedades. Los hallazgos reportados podrían usarse para mejorar el manejo post-cosecha de la carambola.

**Carambola, lyophilization, post-harvest ripening**

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Introduction

Carambola (*Averrhoa carambola* L.), an important member of the family Oxalidaceae, is a tropical shrub introduced in Mexico. The site of origin is not clearly defined. However, it is considered endemic to Indonesia (Nakasone and Paull, 1999; Orduz and Rangel, 2002) or Malaysia and Indonesia (Zeven and De Wet, 1982; Watson et al., 1988). It is distributed in Mexico, at tropical and sub-tropical regions, mainly in the states of Colima, Chiapas, Guerrero, Michoacán, Morelos, Nayarit, Sinaloa, Jalisco, Tabasco and Veracruz. Here, two introduced varieties are mostly cultivated: Golden Star and Arkin, (Romero-Gómezcaña, 2001; Aundón, 2006; Cruz and Garza, 2006). Having originated in Florida, USA, they both have a medium size and a color palette in which yellow and orange tones predominate. However, Arkin is characterized for having a mayor sugar content and less acidity than Golden Star (Campbell et al., 1985; Morton, 1987; Knight, 1989; Lamberts and Crane, 1990; Knight Jr. and Crane, 2002).

The fruit is very attractive because of its organoleptic, energetic and nutritional characteristics, which favor its traditional consumption. It can be consumed as fresh fruit or as processed as juice, although it may also be used to make fresh or fermented beverages, jelly, pastries, preserves, or dehydrated powders, among others. This flexibility confers a high economic potential to these fruits (Lim, 2012). Diverse medical properties are attributed to them, mainly related to antioxidant, hypoglycemic and anti-inflammatory properties (Dembitsky et al., 2011; Lim, 2012, Pantaleón-Velasco et al., 2014). For example, it is used in Western Mexico as an empiric coadjutant for the treatment of Diabetes Mellitus type II (MD2). Their recognized hypoglycemic activity (Cazarolli et al., 2009; 2012), has great public health potential, considering that MD2 has become a serious worldwide health problem, mainly in developing countries, such as Mexico (Vázquez and Panduro, 2001; Olaz-Fernández et al., 2006; Salcedo et al., 2008; Shamah-Levy et al., 2020).

Studies of the physicochemical characterization of carambola are scarce as well as nutritional information in the context of medical (Calzada, 1980, Pérez-Tello et al., 2001; Tello et al., 2002; Ding et al., 2007; Novillo, 2009).

Besides, the data available in this respect is highly variable, while most recent studies about this fruit, have only focused on the physiological analysis of the different maturity states of the carambola, without specifying the variety (Narain et al., 2001) or have concentrated on the Golden Star variety only (Siller-Cepeda et al., 2004). Respiratory and compositional data, of both fruit varieties, have been generated, but only in fruits harvested at physiological maturity (O’Hare, 1993). Nevertheless, studies designed to determine more precisely the post-harvest behavior of these fruits, are needed, mostly at room temperature conditions and including the two varieties with mayor commercial importance in Mexico and North America, such as Golden Star and Arkin. Therefore, one of the aims of this investigation was to generate physicochemical information of Golden star and Arkin carambola fresh fruits in two well defined post-harvest states: just after harvest, and ten days post-harvest, at room temperature. It is worth noting that in this investigation, the fruits analyzed were collected directly in a plantation located in the state of Jalisco, unlike many other studies, that have used fruit purchased in markets or convenience stores. Also, a physicochemical characterization of lyophilized fruits of these two varieties was performed in the two post-harvest periods mentioned. The latter based on findings that have reported that this drying process is capable of affecting the composition, and/or the biological activity of various plant tissues or plant-derived extracts, including the carambola fruit (Shofian et al., 2011). The information proportioned in this study contributes to a more defined knowledge of the post-harvest behavior at room temperature of these important Carambola fruit varieties and provides information regarding the effect that lyophilization has on the levels of important nutritional components. The data proportioned could serve as a guide for the correct post-harvest management of these varieties at room temperature. Also they suggest that the pointed differences between the studied varieties, their differing post-harvest storage behavior or the lyophilization effect, could have implications in their medical use, mainly as an antioxidant, hypoglycemic or anti-inflammatory agent.
Materials and methods

Description of the study site, sample collection and preparation of plant material

For the collection of plant material, the phenology at maturation time, harvest and ingestion was considered. The fruits selected for analysis were those having a mature appearance, with the predominance of golden-yellow pulp color. A total of 120 carambola fruits, var. Golden Star and Arkin, were collected randomly in a commercial plantation established in the municipality of Cihuatlan, Jalisco, located at southwest end of the state (19° 22’ 30” N, 104° 42’ 30” W, at 13 m.a.s.l). The fruits were transported cold to the laboratory. Once in the laboratory, the fruit specimens chosen to determine the post-harvest effect were kept at room temperature during 10 days, before being stored at -20 °C. Thirty Golden Star and 30 Arkin fruits were used for each time of storage, i.e., immediately after harvest (IH-0) and after a 10-day post-harvest period (PH-10). Fruits were washed and blended in an electric blender (Taurus-Group, Barcelona, Spain). The crushed product was collected and stored at -20 °C, for their latter physicochemical analysis, which was performed after a maximum period of 10 days. Part of the triturated tissue was freeze-dried using a Labconco 77520 lyophilizer (Labconco, Kansas City, MO, USA).

Determination of size and weight of the fruit

The size of the collected fruits, along the longitudinal and radial axis, was measured using a millimetric Vernier caliper. The individual weight of each fruit (in g), was registered in an analytical balance (Ohaus, Parsippany, NJ, USA).

Determination of the Humidity content

For the determination of the humidity content, the fresh weight of the complete fruits was registered. Then, they were dried at a constant temperature of 130 °C during 48 h. The percentage of humidity was calculated by the method of Nollet (1996), taking into consideration the lost weight of the sample during drying.

Determination of pH, Total Soluble Solids content (TSS) and acidity (%)

The pH was measured immediately after crushing of the carambola fruit, using a manual pH-EC-TOS METER potentiometer (Hanna Instruments, Woonsocket, RI, USA). The Total Soluble Solids content (TSS) was determined using the method described by the Association of Official Analytical Chemistry (AOAC, 1999). Briefly, 0.3 g of carambola fruit tissues were weighed to prepare an aqueous extract (1:5 w/v), aliquots of which were placed in a manual electronic refractometer, (Atago Pocket Refractometer PAL-1 (0–53%/ 1000mL; Atago, Tokyo, Japan). The TSS were expressed as percentage in °Brix. The percentage of acidity was determined by the method described by the Association of Official Analytical Chemistry (AOAC, 1999), except that the acidity of the aqueous extracts was determined using a manual electronic refractometer, (Atago Pocket Acidity Meter: Citric Acid; Atago PAL ACID-1), range 1~40g/1000mL; Atago Tokyo, Japan).

Extraction and determination of the protein content

The protein content was determined using the Bradford method (1976), which is based on the interaction of the Comassie G250 blue pigment with proteins. Thus, aqueous extracts were prepared, using 20 mg of fresh and 50 mg of lyophilized tissues, due to the different characteristics of the samples tissue, in 400 μL of distilled water. These were stirred for 10 min. at 4 °C and centrifuged at 12,000 rpm for 10 min. Ten μL aliquots of the supernatant were added to 200 μL of the Bradford reactive and the color produced was read at 465 nm.

Determination of Starch content

For starch content determinations the Wright et al. (1998) and Geigenberger et al. (1998), methods were employed, with some modifications. To 200 mg of fresh tissue or 50 mg of lyophilized tissue, 500 μL of extraction buffer was added; the mix was stirred and centrifuged at 12000 rpm for 10 min. The supernatant was collected, and the pellet was extracted two more times, repeating the above steps.
The combined supernatants were dried in a vacuum concentrator system Maxi-dry (Maxi-Dry Lyo; Heto-Holten, Denmark) during 24 h and suspended in 500 µL of Hepes buffer, pH 7.5 for the determination of soluble NSCs (see below). The starch-containing pellet was suspended in 500 µL of distilled water and incubated for 2 h in a water bath at 95 °C. Later, the tubes were covered with aluminum foil, and were allowed to cool for 5 min. Then, 500 µL of the reactive solution was added (0.8 mg α-amylase type VI-B from porcine pancreas [Sigma-Aldrich, St. Louis, MO, USA] and 0.34 mg de amylglucosidase from Aspergillus niger [Sigma], in 50 mM Hepes buffer, pH 7.5) and incubated in a water bath at 55 °C during 24 h. The reactive mixture was centrifuged at 12000 rpm for 10 min, and aliquots of 10 to 15 µL were taken from the supernatant, which were placed in a 96-well microplate, adjusting the volume with 50 µL of distilled water. The plate was incubated at room temperature during 45 min and the absorbance was read at 460 nm. The content of starch is expressed in µmol eq of glucose/ g DW.

Extraction and determination of glucose, fructose and sucrose

The samples derived from the extraction of starch, were diluted 1: 20 v/ v and 5 to 10 µL aliquots were placed in a 96-well microplate. Then, 10 µL of extract and 200 µL of the reactive solution for sugars (0.1 mM NADP+, 0.2 mM ATP, glucose-6-phosphate-dehydrogenase [G6PDH, from yeast grade II, Roche Life Science, Indianapolis, IN, USA] and 0.1 mM NADP+, 0.2 mM ATP, glucose-6-phosphate-dehydrogenase [G6PDH, from yeast grade II, Roche Life Science, Indianapolis, IN, USA] in 50 mM Hepes buffer, pH 7.5) were combined. The plate was stirred 10 seconds and 5 absorbance readings at 340 nm were realized (This is the blank absorbance). The glucose determination was performed adding 2 µL de hexokinase (Roche) after which the absorbance read at 340 nm each two min., till the absorbance value was constant. For the determination of fructose, the plate was removed from the lector and in each pool 2 µL of phosphoglucose-isomerase from yeast (Roche) was added and the absorbance read at 340 nm each two min., till the value of absorbance was constant. Finally, for the determination of sucrose, the plate was again removed from the lector and in each pool 4 µL of invertase was added (Invertase grade VII, from Bakers yeast, Sigma). Each of these non-structural carbohydrates was expressed in µmol/ g DW.

Determination of the amylolytic and polygalacturonase activity

Both activities were measured in vitro using lyophilized tissues from carambola fruits corresponding to the IH-0 and PH-10 post-harvest treatments, respectively. The amylolytic activity was measured according to the Bernfeld (1955), while the polygalacturonase activity was determined using the method described by Gross (1982). Both methods were modified for their use in microplates.

Results

Physical characterization of carambola fresh fruit and after storage at room temperature

Notable differences in the physical parameters determined in the two carambola varieties were detected. In fruits analyzed immediately after harvest (equivalent to the point of maturity, when the transition of color goes from green to yellow) or IH-0, the average weight of the Golden Star (GS) and Arkin (Ar) varieties were 91.93 g and 75.01 g, respectively. However, a notorious weight-loss occurred in GS fruits analyzed 10-days post-harvest at room temperature, or PH-10. This effect was not so pronounced in Ar fruits (Table 1). A similar tendency was observed in the fruit size measures made, although the differences were not so marked between the IH-0 and PH-10 treatments.

Compared to AR, the longitudinal axis at IH-0 was larger in GS fruits. However, and similar to the above parameter, longitudinal fruit size was considerably reduced in the GS variety at PH-10, but not in Ar fruits (Table 1). A similar pattern was observed when the radial axis the of the fruits of both varieties was measured at PH-10. Thus, this parameter was reduced significantly in GS, but not in Ar, fruits (Table 1). Conversely, no differences in the longitudinal and radial axes the of the fruits of both varieties subjected to IH-0 were detected. On average, the fruit of both varieties (at both times of harvest), presented a diameter of 4.94 cm, a longitude of 8.31 cm and a weight of 67.84 g. The data indicated on Table 1 also show that the humidity content in GS fruits remained constant between stages IH-0 and PH-10, whereas in Ar fruits it changed significantly.
The activity of polygalacturonase enzymes, capable of softening the fruit cell walls, is shown in Graphic 1A. It indicates that a significant increase in polygalacturonase activity was similarly produced in GS and Ar fruits subjected to the PH-10 treatment. This finding discards the possibility that the differences observed in the physical integrity of the fruits of both varieties were related to loss of cell wall integrity factor. Nevertheless, a more extensive and detailed study of other cell-wall related enzymatic activities is suggested in future carambola fruit experiments. The latter, considering the obvious physical changes that the fruits of the GS and Ar varieties underwent at the different post-harvest times examined.

Chemical characterization in carambola fresh fruit and the effect of storage at room temperature

No significant differences in the pH of fresh fruits of the GS and Ar varieties was observed at IH-0. However, the pH was slightly reduced, by approximately 0.5 units, in fruits of both varieties subjected to PH-10 (Table 2). The acidity content remained stable in GS fruits, while it increased in PH-10 Ar fruits (Table 2), thereby coinciding with the pH values detected. The TSS concentration (in °Brix) was similar in both varieties of fruit at IH-0. However, at PH-10 the TSS increased significantly in Ar fruits, while they decreased slightly in GS fruits (Table 2).

Differences in protein content were also observed. Higher values were detected in Ar fruits at IH-0. However, a 21% decrease was produced at PH-10 while a contrary behavior was observed in GS fruits, where a 10% increase in protein concentration was observed (Table 2). Compared to Ar fruits, the average starch contents were significantly higher in GS fruits at IH-0. These were not modified by the PH-10 treatment (Table 2). The results displayed in Graphic 1B suggest that the differences in starch levels observed between these two varieties could be associated with differences in amyloitic activity levels, which were lower in GS fruits, predominantly at PH-10. In addition, the PH-10 treatment also significantly affected the levels of soluble NSCs that, in general, tended to increase, except for sucrose, whose levels increased slightly in GS and not at all in Ar. Most noteworthy was the 1.7-fold increase in glucose levels observed in GS fruits after PH-10 (Table 2).

Effect of drying by lyophilization on the chemical composition of the carambola fruits in two states of maturity

In this work, the effect of lyophilization, or freeze-drying, on the chemical composition of GS and Ar fruits, at IH-0 and PH-10, was also studied. As shown in Table 3, this process had a drastic effect on all components analyzed, which was dependent on both the fruit variety and storage treatment. In GS fruits, the protein levels decreased markedly in lyophilized tissue predominantly at PH-10, where the reduction of protein content was almost of 75% lower in the lyophilized tissues. The lyophilization effect on the protein content in Ar fruits was only observed at PH-10, where a 40% reduction in the protein content of lyophilized tissues was observed (Table 3).

A drastic fall was also detected on the starch levels of, particularly in GS fruits and especially, at PH-10. A similar tendency was observed for Ar fruits (Table 3). In contrast, the freeze-drying process drastically increased the soluble NSCs content, which registered a ca. 10- to 15-fold increase of the levels detected in fresh fruit tissues. The effects on NSCs content observed depended on the NSC type, the fruit variety and the post-harvest treatment. In this regard, glucose and fructose content in Ar followed the same pattern observed in fresh tissue, where the highest values were detected at PH-10.

Discussion

Physical characterization of fresh fruits (IH-0) and after storage at room temperature (PH-10)

Carambola GS fruits retained their characteristic ovoid or ellipsoidal shape despite having undergone the most drastic changes in the axial axis as a result of the post-harvest period imposed (Daza et al., 1998; Cubillos and Isaza, 1999; González, 2000). This effect was probably due to the predominance of the axial longitude over the radial longitude, as observed in Table 1. However, it is important to mention, that the longitude of the GS fruits examined in the present study was superior to the one registered by other authors, where the axial longitude was between 6 to 12 cm, and the radial, from 3 to 6 cm (León, 2000).
In addition, the longitudinal and radial axes reported by Palacios and Rodríguez (2001) were 7.46 cm and 4.56 cm, respectively similar to those found by Siller-Cepeda et al. (2004). The average diameter (4.9 cm), longitude (8.3 cm) and weight (67.84 g) of the GS and Ar fruits were similar to those reported by Narain et al. (2001), but contrary to the data presented by Pérez-Barraza et al. (2005).

The marked differences in fresh weight, in addition to the dimensional changes of the GS fruits that occurred at PH-10 could have been a direct consequence of their severe dehydration, considering that carambola fruits are known to suffer significant water losses when stored at room temperature. For example, Pérez-Tello et al. (2001) reported considerable water losses in fruits of the “Yau” variety, stored for 30 days in controlled environment chambers at 20 ºC from day five on.

The maintenance of water content in carambola fruits is considered an important post-harvest quality factor, since dehydration can lead to a surface browning produced by the oxidation of the epidermal cells of the fruit (Nakasone and Paull, 1999). For this reason, they are normally covered with a wax coat or a polyethylene film, or are maintained under refrigeration. These precautions are designed to avoid, besides a drastic weight-loss, the generation of undesirable colors or their deformation, particularly due to degradation effects at the edges of the fruits (Vines and Grierson, 1966; Sanchez, 1990; Wiley, 1994; Thompson, 2006).

The drastic changes in weight and the water loss in Ar fruits, could be attributed to fluctuations in polygalacturonase activity levels. However, this factor was discarded, since the results showed a significant increase in polygalacturonase activity (Graph 1A) in both GS and Ar fruits at PH-10. However, the negative effects observed coincided with similarly deleterious post-harvest damages reported in carambola fruits conserved at room temperature (Prado et al., 2005) and in other perishable fruits, like guayaba, (Singh and Chauhan, 1982; Patel, 2002; Rodeo et al., 2018) and grapefruit (Castro et al., 1999).

In this respect, it is pertinent to mention that polygalacturonases, related pectinolytic enzymes and other enzymes capable of degrading the cell wall (e.g. celluloses and hemicelluloses) were initially purified and analyzed in carambola fruits of an unspecified variety at an optimum maturity stage (Kwek and Ghazali; 1986; Ghazali and Leong, 1987) and in response to different storage conditions (Chin et al., 1999; Ali et al., 2004). A later study did not detect differences in polygalacturonidase (PG) activity in slices of carambola fruits of the Fwang Tung variety sampled at two different maturity stages (e.g. green-yellow and totally yellow) (Teixeira et al., 2005). However, another subsequent study found that PG activity increased considerably during the storage of fruit slices of the same variety, despite of the use of packages designed to maintain them in a controlled environment (Teixeira et al., 2007), similar to what was previously observed by Chin et al. (1999). In addition, Teixeira et al. (2012), reported the presence of soluble pectin derived from a greater activity of PG and pectolase was identified as one of the components responsible of the reduced quality during storage of carambola fruit slices of an Israeli variety at its commercial maturity point. For the above reasons, it is pertinent to argue that a more thorough analysis of these crucial enzymes in the GS and Ar varieties should be contemplated in future experiments with these carambola fruits. In this context, the activity of these enzymes should be kept at a minimum, contrary to some other applications where active pectolytic enzymes are needed, such as in the clarification of carambola wine (Averrhoa carambola) at the start of the fermentation process or in the production of beer using other crops such as rice (Magadama-Ramírez, 2021). In this respect, the agronomic management of carambola fruit cultivation could be an important factor to be explored, considering a recent study that reported a considerably improved after-harvest life of carambola fruits resulting from the application of calcium hydroxide to the soil. This effect was attributed to their higher calcium levels, which was thought to contribute to the strengthening of their respective cell walls due to the modified structure and solubility of the pectin of the middle lamella that defines their firmness. The benefits of a greater firmness are associated to a minor water loss, a reduced respiration rate and lower susceptibility to infection by opportunistic microbes, (Prado et al., 2005).
Acidity was constant in GS fruits, contrary to Ar fruits in which this parameter increased after harvest. This behavior did not correspond to what has been generally observed during carambola fruit storage. For instance, a storage-related decrease in acidity was observed (Campbell et al., 1987) which, if pronounced, was found to negatively affect the sensorial perception of the fruit, making it tasteless (O'Hare, 1993). The reason(s) of the discrepancy between the acidity data of the present study and those normally reported for carambola fruit, remain(s) to be determined. However, the fact that the acidity levels in carambola fruits can widely vary between individuals is a point to ponder (Wilson et al., 1982; Campbell and Koch, 1989). Also, to consider is that the acidity levels are critically dependent of the harvest time stage, since the organic acid levels in the fruits usually increase in relation to the time spent attached to the tree (Wills et al., 1990; León, 2000). Furthermore, it should be mentioned that the acidity recorded in Ar fruits at PH-10 was an unexpected result, taking into account that this variety is considered to be sweet and to have a less acid character (Knight Jr. and Crane, 2002). In contrast, the slight reduction in acidity detected on GS fruits at PH-10, could be related, at least in part, with the loss by oxidation of vitamin C, (Lee and Kader, 2000), a phenomenon that also coincided with the natural cracking of the fruit.

The increased TSS levels in Ar fruits at PH-10 was comparable to findings in other studies (Daza et al., 1998; Tello et al., 2002; Siller-Cepeda et al., 2004; Pérez-Barraza et al., 2005), although the reported increment in TSS during ripening was accompanied by a reduction in acidity (Narain et al., 2001) or, curiously, was affected in oranges by previous application of chitosan (Zapata-Farrofian and Sunción-Guevara, 2021). In this sense, several authors have reported that TSS levels remain almost constant in carambola fruits during storage (Wan and Lam, 1984; Campbell and Koch, 1989; Neog and Mohan, 1991). However, similar to what was observed in this work, variations in the TSS content have been reported to depend on the variety and/or the time elapsed after the harvest (Wagner Jr. et al., 1975; Crane, 1993). It is important to emphasize that the TSS content is an indicative of the sugar content of the fruit and constitutes an important factor influencing the flavor of the juices, jellies, besides being an important quality control characteristic (Knee, 2002).

Storage at room temperature produced changes in the protein content of both GS and Ar fruits at the two storage periods examined. This behavior coincided with reports from Calzada (1980) and Pérez-Tello et al. (2001), in which the protein concentration of ca. 0.5 mg/ mL that was registered in carambola fresh fruits, decreased during maturation, possibly due to a concomitant reduction in the content of peroxidases, polyphenol oxidases and catalases (García et al., 2006). On the other hand, Patil et al. (2010) found that the protein content in carambola fruits increased from 0.65% (in “young” fruits) to 0.85% (in “mature” fruits), a finding that suggested that the protein concentration could vary in relation to the time of harvest and time spent in storage. In relation to other fruit species, the protein content detected in carambola fruits analyzed in this study was slightly lower than those reported in guava (Soria et al., 2003; Athayde Uchôa-thomaz et al., 2014), apple (Bordeleau et al., 2002), pineapple (Krayn, 2006) and orange (Rodrigo and Zacarias, 2007). Notwithstanding, they were higher than those found in babaco (Araujo-Ramírez, 2021).

The high content of starch in GS fruit was probably to reflection of the reduced sweetness that these fruits have in comparison to Ar fruits. Increased starch levels have also been associated with a sandier texture.
The NSCs levels were found not to be significantly affected at PH-10, except for glucose, in GS, where it underwent an approximately 25% reduction, while the sucrose levels in Ar fruits remained unchanged. The latter information regarding Ar fruits, was in agreement with the reported literature (Campbell et al., 1987). Conversely, the sucrose levels, in GS fruits at the PH-10 stage, which could have influenced the sweetness of the fruit, showed a very similar tendency to the one observed by Pérez-Tello et al. (2001) in carambola fruits stored at 20 °C during 30 days. In addition, the fructose levels in Ar fruits, that generally are 1.5 times higher than those in GS fruits, a difference that determines to some extent the difference in sweetness between both varieties (Campbell et al., 1987; Campbell and Koch, 1989), were significantly higher in Ar fruit at IH-0. On the other hand, this difference in NSCs (i.e., higher in GS fruits), was lost, mainly in terms of glucose, in GS fruits at PH-10. This stark modification suggests the possible activation of an active isomerization process in GS fruits during storage, which should be experimentally confirmed. In contrast, the possibility that the observed changes in glucose and fructose levels in both varieties were due to an increment in sucrolytic activity by invertases can be discarded considering that the sucrose levels in these fruits were increased or remained constant.

This scenario was contrary to the one suggested by other authors (Pérez-Tello et al., 2001). However, the results obtained in this study agreed with a proposal suggesting that the rise in glucose and fructose during the storage of carambola fruits may be due to the differential use of these sugars for respiration. Anyway, an aspect that remains to be defined is the origin of the increased levels of NSCs, except starch, observed in fruits of both varieties at PH-10. It is tempting to speculate that these generated by a process of gluconeogenesis from free amino acids, or inclusive fatty acids. This proposal is somewhat supported by a proteomic analysis of the maturation process of apple, in which an accumulation of enzymes involved in the gluconeogenic process were found (e.g., malic enzyme dependent of NADP+ and triose phosphate isomerase; Shi et al., 2014), similar to what was previously reported in the maturation process of tomato (Goodenough et al., 1985).

Effect of the drying by lyophilization in the chemical composition of carambola fruit in two states of maturation

The increase in the levels of NSCs, except starch, could be simply due to a process of concentration caused by the loss of great part of the moisture of the fruit that, as reported in Table 1, was higher than 90%. However, it may not to be discarded that during lyophilization, the hydrolysis of starch was activated, as supported by the drastically decrease in carambola fruit starch levels already mentioned, from which the rise in soluble NSCs could have been generated. This is a question raised by the results of this study that will require a posterior analysis to be validated or rejected.

However, the changes caused by freeze-drying observed in this study are in accordance with the findings of previous reports in which the lyophilization process was found to cause numerous changes in the composition of plant tissues of diverse type. Included among these, are the observed modification of total phenolic compounds content and antioxidant activity the levels in tropical fruits, including carambola (Shofian et al., 2011). Likewise, other investigations studied the effect of this process on the integrity of plant tissues (Chang et al., 2006) and found evidence suggesting that the observed changes occurring as a result of lyophilization, could be due to a perturbation of the integrity of the fruit cells, leading to a de-compartmentalization of enzymes, substrates and activators that promoted the degradation of certain phenolic compounds. In addition, studies realized with onions at different stages of long term storage, including the lyophilization, found that the levels of flavonoids could raise, decrease or remain unaltered depending on the type of post-harvest process they were summited to and also on pre-storage manipulation they received (Amarowicz et al., 2009; Pérez-Gregorio et al., 2011a; Pérez-Gregorio et al., 2011b).
Table 1 Physical parameters (fresh weight, radial longitude and axis and percentage of humidity) in carambola (Averrhoa carambola L.) fresh fruits, varieties Golden Star (GS) and Arkin (Ar), immediately after harvest (cero days; IH-0) and after a 10-day post-harvest period (PH-10). The data are means ± s.e. (n = 30). Different letters within each column indicate statistically significant differences between the two varieties at P < 0.05 for the different measurement dates.

<table>
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<th>Variables</th>
<th>GS</th>
<th>IH-0</th>
<th>PH-10</th>
<th>Ar</th>
<th>IH-0</th>
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<tbody>
<tr>
<td>Fresh weight (g)</td>
<td>93.9 ± 1.11a</td>
<td>95.1 ± 1.64a</td>
<td>94.1 ± 1.59a</td>
<td>94.8 ± 1.37a</td>
<td>94.8 ± 1.37a</td>
<td>94.8 ± 1.37a</td>
</tr>
<tr>
<td>Radial longitude (cm)</td>
<td>5.53 ± 0.42a</td>
<td>5.53 ± 0.42a</td>
<td>5.53 ± 0.42a</td>
<td>5.53 ± 0.42a</td>
<td>5.53 ± 0.42a</td>
<td>5.53 ± 0.42a</td>
</tr>
<tr>
<td>Axis longitude (cm)</td>
<td>9.02 ± 0.87a</td>
<td>8.96 ± 0.71a</td>
<td>8.86 ± 0.72a</td>
<td>8.86 ± 0.71a</td>
<td>8.86 ± 0.72a</td>
<td>8.86 ± 0.71a</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>97.90 ± 0.89a</td>
<td>95.40 ± 1.79a</td>
<td>97.00 ± 2.36a</td>
<td>98.10 ± 2.59a</td>
<td>98.10 ± 2.59a</td>
<td>98.10 ± 2.59a</td>
</tr>
</tbody>
</table>

Table 2 Chemical characterization in carambola (Averrhoa carambola L.) fruits, varieties Golden Star (GS) and Arkin (Ar), determined immediately after harvest (cero days; IH-0) and after a 10-day post-harvest period (PH-10). The results are means ± s.e. (n = 30). 1 Starch levels are represented as µmol eq of glucose/g DW.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GS</th>
<th>IH-0</th>
<th>PH-10</th>
<th>Ar</th>
<th>IH-0</th>
<th>PH-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose (µmol/g DW)</td>
<td>2.28 ± 0.24a</td>
<td>2.54 ± 0.28a</td>
<td>2.54 ± 0.28a</td>
<td>2.54 ± 0.28a</td>
<td>2.54 ± 0.28a</td>
<td>2.54 ± 0.28a</td>
</tr>
<tr>
<td>Fructose (µmol/g DW)</td>
<td>3.26 ± 0.73a</td>
<td>3.93 ± 0.83a</td>
<td>3.93 ± 0.83a</td>
<td>3.93 ± 0.83a</td>
<td>3.93 ± 0.83a</td>
<td>3.93 ± 0.83a</td>
</tr>
<tr>
<td>Glucose (µmol/g DW)</td>
<td>3.80 ± 0.71a</td>
<td>4.41 ± 0.80a</td>
<td>4.41 ± 0.80a</td>
<td>4.41 ± 0.80a</td>
<td>4.41 ± 0.80a</td>
<td>4.41 ± 0.80a</td>
</tr>
<tr>
<td>Protein (mg/mL)</td>
<td>5.23 ± 0.22a</td>
<td>6.08 ± 0.20a</td>
<td>6.08 ± 0.20a</td>
<td>6.08 ± 0.20a</td>
<td>6.08 ± 0.20a</td>
<td>6.08 ± 0.20a</td>
</tr>
<tr>
<td>TSS (°Brix)</td>
<td>15.45 ± 2.88a</td>
<td>16.70 ± 2.40a</td>
<td>16.70 ± 2.40a</td>
<td>16.70 ± 2.40a</td>
<td>16.70 ± 2.40a</td>
<td>16.70 ± 2.40a</td>
</tr>
<tr>
<td>Acidity</td>
<td>15.75 ± 0.27a</td>
<td>15.75 ± 0.27a</td>
<td>15.75 ± 0.27a</td>
<td>15.75 ± 0.27a</td>
<td>15.75 ± 0.27a</td>
<td>15.75 ± 0.27a</td>
</tr>
<tr>
<td>pH</td>
<td>2.85 ± 0.62a</td>
<td>2.32 ± 0.07a</td>
<td>2.32 ± 0.07a</td>
<td>2.32 ± 0.07a</td>
<td>2.32 ± 0.07a</td>
<td>2.32 ± 0.07a</td>
</tr>
<tr>
<td>Starch (ºG)</td>
<td>14.20 ± 1.29a</td>
<td>10.71 ± 2.33a</td>
<td>10.71 ± 2.33a</td>
<td>10.71 ± 2.33a</td>
<td>10.71 ± 2.33a</td>
<td>10.71 ± 2.33a</td>
</tr>
</tbody>
</table>

Table 3 Chemical characterization lyophilized carambola (Averrhoa carambola L.) fruits, varieties Golden Star (GS) and Arkin (Ar), processed immediately after harvest (cero days; IH-0) or after a 10-days post-harvest period (PH-10). ¹Data are means ± s.e. (n = 30). ²Reported in µmol equivalent of glucose/g DW.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GS</th>
<th>IH-0</th>
<th>PH-10</th>
<th>Ar</th>
<th>IH-0</th>
<th>PH-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar (ºG)</td>
<td>0.16 ± 0.06a</td>
<td>0.16 ± 0.06a</td>
<td>0.16 ± 0.06a</td>
<td>0.16 ± 0.06a</td>
<td>0.16 ± 0.06a</td>
<td>0.16 ± 0.06a</td>
</tr>
<tr>
<td>Pectin (ºG)</td>
<td>7.07 ± 0.55a</td>
<td>7.07 ± 0.55a</td>
<td>7.07 ± 0.55a</td>
<td>7.07 ± 0.55a</td>
<td>7.07 ± 0.55a</td>
<td>7.07 ± 0.55a</td>
</tr>
<tr>
<td>Starch (ºG)</td>
<td>1.16 ± 0.01a</td>
<td>1.16 ± 0.01a</td>
<td>1.16 ± 0.01a</td>
<td>1.16 ± 0.01a</td>
<td>1.16 ± 0.01a</td>
<td>1.16 ± 0.01a</td>
</tr>
</tbody>
</table>

Graphic 1 Levels of pectinolytic (A) and amilolytic (B) activity determined in carambola fruits, varieties Golden Star (GS) and Arkin (Ar), sampled at two states of maturity: IH-0, processed immediately after harvest at day 0 and representing fruits at physiological maturity, and PH-10, representing fruits harvested at physiological maturity and later stored at room temperature during 10 days. Each bar represents the means ± s.e. of measures performed using a total of 10 individual fruits. Different letters over the bars represent significantly different values (P < 0.05) determined by an ANOVA followed by a Tukey-Kramer test.

Acknowledgments

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Conclusions

The following can be concluded based on the results obtained: i) the two more important commercial varieties of the carambola fruit, at least in the American continent, showed a contrasting behavior in terms of their physicochemical composition and in the way it was altered at two maturity stages. This finding reinforces reported data from previous studies in which a marked varietal effect is described regarding diverse aspects associated with post-harvest storage, such as browning by oxidation; ii) the divergent physicochemical characteristics of the varieties studied, that frequently differed from published data, as well as their contrasting post-harvest behavior, could have been affected by undetermined factors related to the cultivation site and/or to the agronomic practices employed for their production, and iii) the drying by lyophilization had a very marked effect on the protein and NSC contents of the carambola fruits. The effect was also dependent on the variety and on the maturity state of the tissue employed for the freeze-drying process. These aspects suggest that both short-term storage at room temperature, as well as drying by lyophilization, affect, in a contrasting way, not only the physicochemical composition of the fruits but, very probably also, their biological activity. The latter aspect is important considering the wide potential for anthropogenic use offered by carambola fruits.

References


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