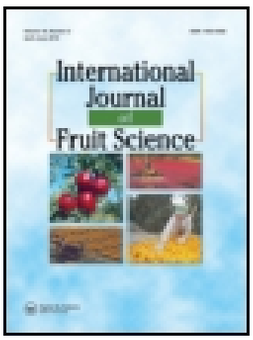

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Organic Compounds Determined at Different Levels of Ripening of the Pineapple (*Ananas comosus L. Merr.*) Cv Cayenne in Two Cultivation Systems under Subtropical Conditions

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ABSTRACT

This article studies the organic compounds of pineapple fruit, during ripening in two cultivation systems (greenhouse and field), in a subtropical region. Total reducing sugars, organic acids, total phenolic content and antioxidant activity were measured during fruit growth (60, 90, 120 and 150 days after full bloom). In addition, leaf ¨D¨ was used for determination of chlorophyll content, pH and malic acid in three moments of the day during the same cycle. The concentrations of reducing sugars, total phenolic content and acidity increased at the end of the growth of the pineapple fruits in both cultivation systems. Field conditions were more favorable for the accumulation of reducing sugars and total phenolic content in fruits.

KEYWORDS

Field; fruit; greenhouse; phenolic content; sugar

Introduction

Pineapple [*Ananas comosus* (L.) Merr.] is a highly valued fruit, and considerable quantities are exported from tropical production countries to the European and North American markets (FAO, 2016).

Pineapple from cultivar 'Smooth cayenne' is distributed worldwide and normally has a pale, yellowish flesh and a brown orange shell at commercial maturity. Pineapples, as other fruits, are an important source of sugars, organic acids, essential minerals, fiber and vitamins for human nutrition and sweetness is well known and appreciated by consumers (Lobo and Paull, 2017). Pineapple juice contains ascorbic acid and is a good source of Vitamin C. Ascorbic acid or vitamin C is an effective antioxidant that also fights against bacterial, viral infections and helps the body absorb iron (Farid et al., 2015). Considering these characteristics, consumers are nowadays more concerned with the nutritional qualities of what they eat such as phenolic

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content and antioxidant activity (Brat et al., 2004). The final quality and chemical composition of pineapple fruit are influenced by the stage of maturation, agronomic and environmental factors (Leonel et al., 2014). Maturation of fruit or other plant tissues involves a series of complex reactions that leads to changes in the phytochemistry of the plants. Distinct phenomena of change in chemical composition such as phenolic contents have been observed during maturation (Yang et al., 2011).

The main limiting factor for the cultivation of pineapple in subtropical regions is low air temperature. Although the plant resists mild and short frosts down to −3°C, its growth is delayed by low air temperatures and stops, depending on the cultivar, at air temperatures between 10°C and 16°C. According to Cunha (1999), the ideal air temperature for the growth and development of roots and leaves of pineapple is between 22°C and 32°C; at predominantly low air temperatures, growth is reduced.

One possibility for this crop in the subtropics is the use of greenhouses; however, environmental conditions generated by the greenhouse system could affect growth and development of plants grown in these conditions, as well as the evolution of the compounds of the fruit (Lobo and Paull, 2017).

Differences in growth and exomorphological characteristics of the pineapple leaves have been detected in field and greenhouse cropping systems (Ebel et al., 2016). Nevertheless, these findings raise a number of unknowns as to whether these morpho-anatomical differences would also be reflected in the photosynthetic activity affecting growth and development of plants and the content of organic compounds in fruits.

In this context, the aim of this work was to study the organic compounds variation of the fruit at different levels of ripening (*Ananas comosus*) in two cultivation systems, greenhouse and field conditions, in a subtropical region in the northeast of Argentina.

Materials and Methods

The experiments were performed at the Estación Experimental de la Facultad de Ciencias Agrarias de la Universidad Nacional del Nordeste, Corrientes Argentina, 27° 28′ 27″ S., 58° 47′ 00″ W; 70 above sea level from 2015 to 2017.

The soil at the experimental site has been classified as Dystric Arenosol according to the World Reference Base for Soil Resources. The relief of the area is gently undulating, with slopes of 1% to 1.5%. The climate is subtropical or mesothermal with an average annual rainfall of 1300 mm, and an annual average air temperature of 21.6°C; it has a well-defined frost-free period of 340 to 360 days. Average annual air temperature is around 21.5°C, the average air temperature of the coldest month ranges between 13°C and 16°C and the average temperature of the warmest month is between 26°C and 27°C; annual

variation is scarce. In summer, absolute maximums air temperatures reach 46.5° C, absolute minimum air temperatures are −1°C to −5.5°C (Escobar, 1996).

The rainfall pattern is characterized by abundance and frequent rainfall exceeding 1,500 mm per year. The main feature of this system is the seasonal irregularity in rainfall distribution, with autumn being the rainiest and winter the driest season.

In this study, we used the pineapple cultivar "Smooth Cayenne". Two experimental batches, one under field conditions and other under greenhouse, were established and drip-irrigated. The plots consisted of two sowing beds with a density equivalent to 74,000 plants per hectare; beds distance from center to center was 1.80 m, with a length of 8.40 m. In each bed, there were four rows of plants, with a spacing of 0.3 m between plants and rows; the two central rows were considered as useful plots (96 plants) to avoid edge effect.

Experimental Design and Sample Collected

The experimental design was randomized with two treatments and three replicates per sample; the experimental unit was the plant. During the fruit growth cycle, fruits and "D" leaves were collected at different phenological stages (60, 90, 120 and 150 days after full bloom). The "D" leaves were collected at three times during the day (sunrise, midday and sunset) for every phenological stages.

Analysis of Plant Material

Fruits were sampled during fruit growth (60, 90, 120 and 150 DAFB). The pineapple fruits were cleaned, peeled off, cut into small slices and homogenized in a blender in a Smart-tek SC 2031 (Smart-tek, Buenos Aires, Argentina). The pulp was stored at −24°C in plastic bags with hermetic closure (Ziploc ®, S. C. Johnson & Son Incorporation, USA).

Total Reducing Sugars

Total reducing sugars were determined using the spectrophotometric method proposed by Carranza et al. (1978). The sample was mixed with an alkaline ferricyanide solution. It was heated until boiling for 10 min and neutralized with 10 ml of 2 N H_2SO_4. Then 4 ml of the arsenomolybdate solution was added. The absorbance was determined at 745 nm using a Spectrophotometer (Jasco V-630-Bio, JASCO Corporation, England).

Acidity

Total titratable acidity was determined by titration to pH 8.1 with 0.05 M NaOH solution and calculated as milligrams of citric acid per 100 g of pulp. (AOAC, 1990).

Antioxidant Activity

The DPPH test (2,2-diphenyl-1-picrylhydrazole) was applied according to Sánchez-Moreno et al. (1998) by reading the absorbances at 517 nm using the spectrophotometer described in 2.2.1.1, and using ascorbic acid as a standard (0.04–0.2 mg ml^{-1}). The results were expressed as mg of ascorbic acid per 100 g of fresh fruit.

Polyphenols

Total phenolic content was quantified using the Folin-Ciocalteau reagent according to the method proposed by Karou et al. (2005). Gallic acid was used as standard (0.05–0.16 mg ml^{-1}) and results were expressed as grams of gallic acid per 100 g of fresh fruits.

The largest fully deployed adult leaf of the plant (leaf ¨D¨) was collected and stored at −24°C.

Total Chlorophyll Content

The Arnon method (Arnon, 1949) was used to determine chlorophyll content. The extraction of chlorophyll was made from 2 g of leaves in a mortar with sand (a tip of spatula) and 15 ml of 80% acetone. From the resulting processed sample, 4 ml were taken and centrifuged (Spectrafuge^{TM} 24DMicrocentrifuge, Labnet, USA) at 10,000 rpm for 2 min. The supernatants were mixed in a 3 ml spectrophotometer cuvette at the time of measurement and the absorbance of the sample was determined at 652 nm using the spectrophotometer described in 2.2.1.1. The value of the specific absorption coefficient for this wavelength was found to be 34.5. The chlorophyll content is determined by applying the Lambert-Beer law using the specific absorption coefficient and the length of the spectrophotometer cuvette.

Malic Acid and pH

The diurnal fluctuation of pH and organic acids (malic acid), characteristic of crassulacean acid metabolism (CAM) plants, is established as the difference between the levels of these determined between the beginning and end of the photoperiod. Therefore, samples of the plant material were collected at sunrise, midday and sunset. In order to determine both parameters, an extraction of the cellular content was obtained by crushing the sample of vegetal material in 20 ml of distilled water. The 20 ml was centrifuged in two reinforced plastic tubes at 3000 rpm for 10 min at room temperature. The supernatants were collected and brought to volume with distilled water to 30 ml. First, the pH of the sample was determined with potentiometer (Metrohm 692 pH/Ion Meter, Metrohm Limited Company, Switzerland) and then the solution was neutralized with 0.05 N NaOH. The determination of organic acid was made by titration; first expressed as μequivalents per g FW^{-1} (fresh weight) of leaf (Kluge and Ting, 1978) and then calculated as

the percentage of malic acid relative to the maximum concentration at sunrise (100%) in the "D" leaf.

Climatic Variables

Maximum and Minimum Air Temperatures and Ambient Relative Humidity

In the greenhouse, measurements were recorded using a data logger (DAF-10 data logger, Schwyz, China) and in the field every 30 min; data were obtained from a meteorological station (OMXH, OMIXOM, Argentina).

Incident PAR

The values of photosynthetically active radiation (PAR) intercepted by the culture were obtained using a ceptometer (Ceptometer BAR-RAD DUAL, Cavadevices Incorporation, Argentina) with PAR radiation sensors, with spectral response in the band between 400 and 700 nm of wavelength. Measurements were performed from eight different points inside the greenhouse and field treatments.

Data Analysis

Prior to comparing the measured variables, the normality of the data (Shapiro–Wills test) and homogeneity of variance were tested. Analysis of variance (ANOVA) and mean comparisons were performed using Duncan's test ($P < .05$). Statistical analyses were carried out using the software INFOSTAT version 2012.

Results

Final concentrations of reducing sugars were 12.23 g 100 g^{-1} and 10.41 g 100 g^{-1} for field and greenhouse, respectively (Figure 1). The variation of total reducing sugars in the greenhouse treatment was scarce at 60, 90 and 120 DAFB. The reducing sugar concentration increased slightly to 120 DAFB, with a strong increase at the end of ripening, peaking at 150 DAFB. Among the stages 120 and 150 DAFB, the concentration of reducing sugars increased 38% and 42% for field and greenhouse, respectively, in respect of the final concentration. The greenhouse's fruits at harvest time (150 DAFB) obtained 14.89% less reducing sugars than the fruits of the field. Significant differences between greenhouse and field-grown plants were found at 60, 120 and 150 DAFB, being higher in-field treatment.

The concentration of citric acid increased as the fruits grew, while the pH behavior was reduced (Figure 2). Significant differences in pH values were found at 60 and 150 DAFB, higher in the field treatment with a final pH 4.09 and 3.95 for field and greenhouse, respectively. The acidity expressed as

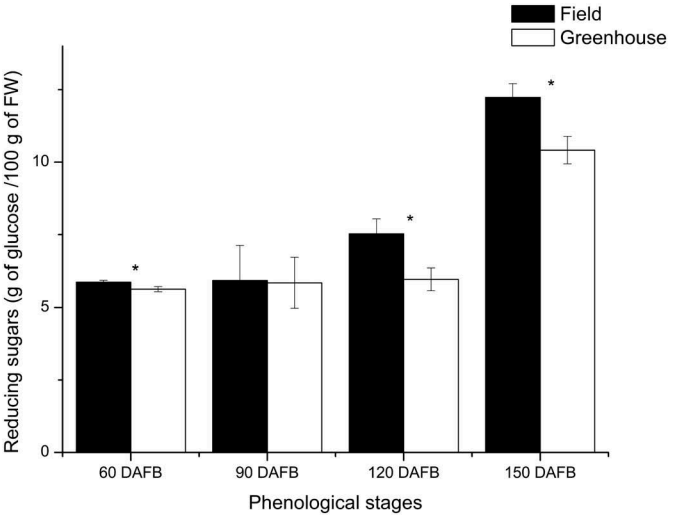

Figure 1. Concentration of reducing sugars in pineapple fruits plants cultivated under field and greenhouse conditions during different phenological stages: 60, 90, 120 and 150 days after full bloom (DAFB). * indicate significant difference at $P < .05$, bars represent standard error of the mean (SEM).

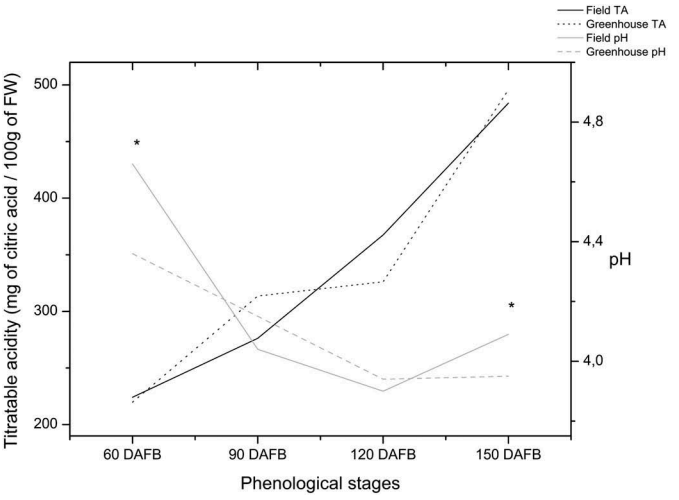

Figure 2. Titratable acidity (TA) and pH in pineapple fruits cultivated under field and greenhouse conditions during different phenological stages: 60, 90, 150 and 150 days after full bloom (DAFB). * indicate significant difference at $P < .05$.

milligrams of citric acid per 100 g fresh weight, showed no significant difference in the citric acid concentration between both treatments during fruit growth. The final concentrations of citric acid were 483.93 mg 100 g^{-1} and 595.81 mg 100 g^{-1} for field and greenhouse, respectively. During fruit's growth, the concentration of citric acid increased 115.90% in field and

125.50% in greenhouse in respect of the initial concentration of citric acid. However, fruits at 90 and 120 DAFB showed quite consistent trends concentrations in the greenhouse treatment at these two phenological stages.

The antioxidant activity expressed as milligrams of ascorbic acid in 100 g of fresh pulp varied from 193.08 mg 100 g^{-1} and 182.24 mg 100 g^{-1} (60 DAFB) to 290.99 mg 100 g^{-1} and 238.87 mg 100 g^{-1} (150 DAFB) for field and greenhouse, respectively (Figure 3). The antioxidant activity showed minimal change during the developmental period at 60 and 90 DAFB, with a consistent rise at 120 DAFB, but there were no significant differences between treatments during fruit growth. At the harvest moment (150 DAFB) the antioxidant activity decreased 63.14 mg 100 g^{-1} in the field and 14.83 mg 100 g^{-1} in the greenhouse, compared with 120 DAFB.

The total phenolic content showed little tendency to change at 60 and 90 DAFB until the 120 DAFB phenological stage, when there was a marked increase in both culture systems with significant differences at 120 and 150 DAFB (Figure 4). As the fruit grew, the total phenolics increased, from 0.41 to 0.75 g of gallic acid equivalents (GAE) 100 g^{-1} fresh weight in the greenhouse and 0.50 to 0.93 g of GAE 100 g^{-1} fresh weight in the field. It is important to highlight that different cultivation system showed influence in total phenolic content.

As expected, the percentage of malic acid decreased during the day and on the contrary, the pH values increased, so the relation between these two variables is inversed (Table 1). Results revealed that between 90 and 150 DAFB the greenhouse treatment metabolized 5–8% more malic acid than the

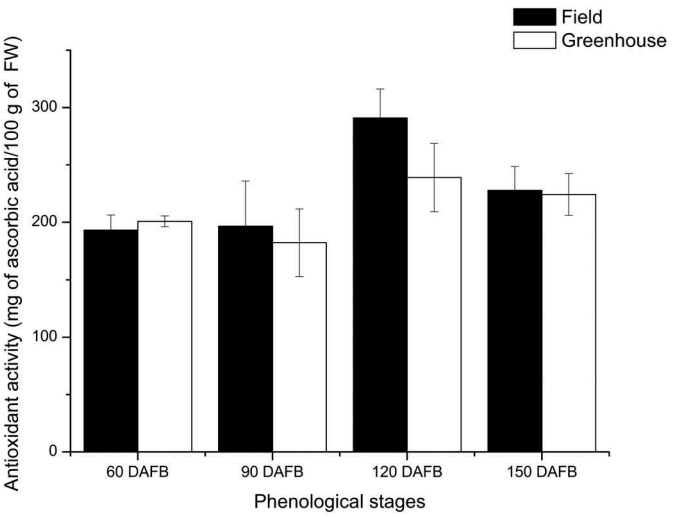

Figure 3. Antioxidant activity of pineapple fruits cultivated under field and greenhouse conditions during different phenological stages: 60, 90, 120 and 150 days after full bloom (DAFB). * indicate significant difference at $P < .05$, bars represent standard error of the mean (SEM).

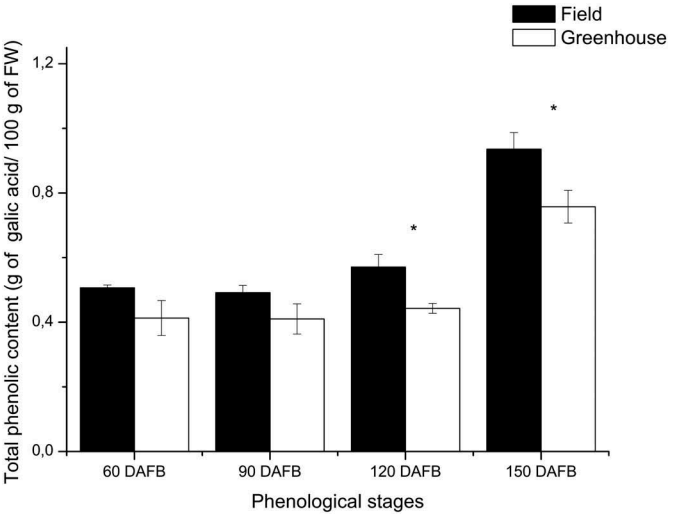

Figure 4. Total phenolic content of pineapple fruits cultivated under field and greenhouse conditions during different phenological stages: 60, 90, 120 and 150 days after full bloom (DAFB). * indicate significant difference at $P < .05$, bars represent standard error of the mean (SEM).

Table 1. Three times daily variations, Sunrise, Midday and Sunset, in malic acid percentage relative to the maximum concentration and pH values of "D" leaves cultivated under field and greenhouse conditions during different phenological stages: 60, 90, 120 and 150 days after full bloom (DAFB). The experiments were performed in Corrientes, Argentina from 2015 to 2017.

		Sunrise		Midday		Sunset	
Phenological stages	Treatments	Malic acid (%)	pH	Malic acid (%)	pH	Malic acid (%)	pH
60 DAFB	Field	100	3.67 a	41.87 a	4.32 a	15.18 a	5.15 a
	Greenhouse	100	3.63 a	42.62 a	4.32 a	15.74 a	5.39 a
90 DAFB	Field	100	3.64 a	25.20 a	4.58 a	19.07 b	5.04 a
	Greenhouse	100	3.72 a	27.70 a	4.58 a	11.55 a	5.52 b
120 DAFB	Field	100	3.53 a	42.12 a	4.04 a	16.44 b	4.95 a
	Greenhouse	100	3.77 a	51.57 b	4.23 a	11.72 a	5.49 b
150 DAFB	Field	100	3.74 b	50.66 a	4.30 a	21.04 b	5.06 a
	Greenhouse	100	3.62 a	42.56 a	4.20 a	14.78 a	5.06 a

Different letters between treatment for each phenological stage indicate significant differences at $P < .05$

field treatment with significant differences. The pH values varied during the day from 3.62 to 5.52 for greenhouse and 3.53 to 5.15 for the field treatment. In order to compare pH values between both treatments during fruit growth, higher values were found in the greenhouse with significant difference at sunset at 90 and 120 DAFB.

During fruit growth, the chlorophyll content in both treatments increased until 90 DAFB and then decreased to the end of the cycle (Figure 5). The lowest values of chlorophyll found in both treatments were at 150 DAFB. There were no significant differences between both treatments since the greenhouse and field plants maintained similar contents throughout the cycle.

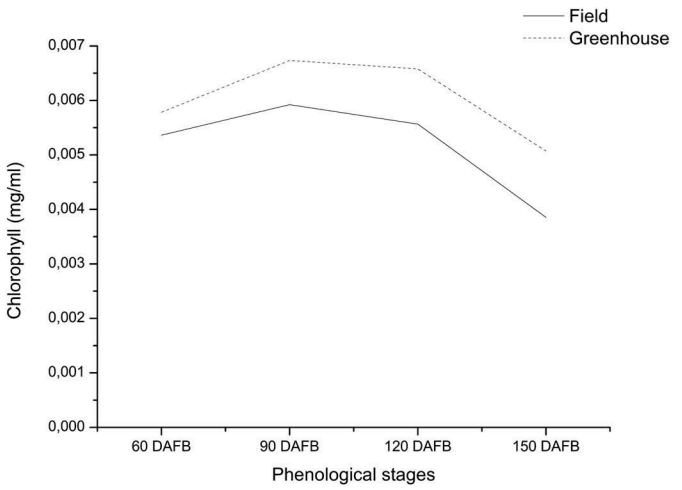

Figure 5. Chlorophyll content in "D" leaves of pineapple plants cultivated under field and greenhouse conditions during different phenological stages: 60, 90, 150 and 150 days after full bloom (DAFB). * indicate significant difference at $P < .05$.

Climate variables were also measured, which included maximum and minimum temperatures, ambient relative humidity and values of photosynthetically active radiation (PAR) intercepted by the crop. The average maximum temperature varied from 35°C to 40°C inside the greenhouse, and 32°C to 34°C in the field (Table 2). The maximum temperature was presented at 60 DAFB in the greenhouse and at 150 DAFB in the field. The differences between treatments in terms of the average minimum temperature were between 1°C and 3°C. In regard to the average maximum humidity (Table 3), it varied from 93.9% to 85.8% inside the greenhouse, and 93.6% to 88.4% in the field. During fruit growth, the difference between average maximum humidity and minimum average humidity was between 49.4% and 53.7% in the field and 40.4% to 48.7% inside the greenhouse. The photosynthetically active radiation (PAR) intercepted by the crop is expressed as μmol of photons m^2 $second^{-1}$ (Table 4). The highest values of radiation for both treatments were 2,306.50 μmol m^2 s^{-1} and 1,784.2 of μmol m^2 s^{-1} for field and greenhouse at 90 DAFB.

Table 2. Maximum and minimum average temperatures obtained in field and under greenhouse treatments during different phenological stages: 60, 90, 120 and 150 days after full bloom (DAFB). The experiments were performed in Corrientes, Argentina from 2015 to 2017.

	Field Max. Temp.(ºC)	Field Min. Temp.(°C)	Greenh. Max. Temp.(ºC)	Greenh. Min. Temp.(°C)
60 DAFB	32.1	20.3	40.1	22.6
90 DAFB	33.1	20.2	38.4	23.6
120 DAFB	33.9	23.1	35.5	25.1
150 DAFB	34.1	23.5	36.2	22.5

Table 3. Maximum and minimum average humidity obtained in field and under greenhouse treatments during different phenological stages: 60, 90, 120 and 150 days after full bloom (DAFB). The experiments were performed in Corrientes, Argentina from 2015 to 2017.

	Field Max. Hum. (%)	Field Min. Hum. (%)	Greenh Max. Hum. (%)	Greenh. Min. Hum. (%)
60 DAFB	93.6%	40.6%	93.9%	46.2%
90 DAFB	94.5%	43.5%	89.3%	42.3%
120 DAFB	88.4%	34.7%	91.6%	42.9%
150 DAFB	89.5%	40.1%	85.8%	45.4%

Table 4. Photosynthetically active radiation intercepted (PARi) in the field and under greenhouse expressed in µmol of photons.m^{2}·$second^{-1}$ during different phenological stages: 60, 90, 120 and 150 days after full bloom (DAFB). The experiments were performed in Corrientes, Argentina from 2015 to 2017.

	Field PARi (µmol.m^{2}.s^{-1})	Greenhouse PARi (µmol.m^{2}.s^{-1})
60 DAFB	2,097.1	1,575,7
90 DAFB	2,306.5	1,784.2
120 DAFB	1,991.2	1,489.5
150 DAFB	1,951.6	1,477.5

These high radiation values correspond to the summer season in this subtropical region.

Discussion

At 120 and 150 DAFB the concentration of reducing sugars increased considerably in both cultivation systems, this increase may be attributed to a greater translocation of photosynthates to the fruit (Hernández et al., 1977). The final content of reducing sugars at 150 DAFB was slightly higher to that obtained by Ramírez et al. (2011) for the same variety; this result is associated with PAR and temperature. In the field system PAR was 25% higher due to the absence of plastic which is a favorable condition for the accumulation of reducing sugars (Sinclair et al., 1999). On the other hand, average maximum temperature inside the greenhouse varied from 35°C to 40°C, and according to Malézieux et al. (1994) high temperatures, above 35° C, affect fruit development.

The highest acidity was found at 150 DAFB, this behavior is due to the fact that in tropical fruits, as is the case of the pineapple, the greater amount of organic acids is synthesized in maturation (Azcon-Bieto et al., 1993). The acidity and pH of mature fruits (150 DAFB) of smooth Cayenne cultivar grown in Corrientes in both cultivation systems agrees with Rebolledo-Martínez et al. (2006). The behavior of these variables is in accordance with Seymour et al. (1993), who states that the pH declines from 3.9 to 3.7 and the concentration of citric acid increases as the fruit approaches the state of total maturity.

Although there are no records in the variation of phenolic concentration during fruit growth in pineapple cv. Smooth Cayenne in different cultivation systems, it has been demonstrated that the total phenolic content of pineapple in field cultivation increases during the same phenological stages (Ding and Syazwani, 2015). In pineapple, these compounds can vary according to the maturation stage of the fruit and even in response to environmental factors and cultivation systems (Kermasha et al., 1987). This study demonstrate that total phenolic content is influenced not only by the maturation stage of the fruit but also in response to different cultivation systems.

The concentration of secondary metabolites was higher during fruit growth, since most of these metabolites are responsible for the plant defense function, while at the maturation stage (150 DAFB) there is an increase in concentration of sugars that agrees with Celli et al. (2011). The antioxidant activity values had a positive and weak correlation with the phenol content detected ($r = 0.24$), which could be due to the presence of other non-phenolic compounds such as carotenoids and ascorbic acid with antioxidant potential (Zapata et al., 2014). The antioxidant activity found at 150 DAFB for both treatments agrees with Duque and Morales (2005), who found values of 289 mg of ascorbic acid 100 g^{-1} of pulp.

The malic acid variation is explained due to the diurnal photosynthetic metabolism. The malic acid is accumulated in the vacuoles during the night and decarboxylated during the day, releasing CO_2 that passed to the C3 or Calvin-Benson photosynthesis cycle (Sale and Neales, 1980). The higher metabolization rate of malic acid during the day in the greenhouse was due to the higher temperatures generated in this cultivation system which improves the action of the enzyme phosphoenolpyruvate carboxylase to fix CO_2.

The greenhouse cultivation benefits the vegetative development including foliar area, fresh weight and dry weight of "D" leaves (Ebel et al., 2016) that agrees with the higher metabolization of the percentage of malic acid in plants grown inside the greenhouse.

Malic acid is cited as the main organic acid that undergoes variation during photosynthesis; however other acids such as citrate and succinate also play a role in this CAM metabolism (Kenyon et al., 1985). These acids would explain the significant differences found in pH at the end of the day when there were no significant differences in the concentration of malic acid.

The lowest value of chlorophyll for both treatments was found at 150 DAFB probably due to the senescence of the fruit and yellowing of the leaves at the end of ripening (Morales et al., 2001). The behavior in the chlorophyll content in the pineapple plants during its growth is expected for this species (Ebel et al., 2016).

Even though temperatures inside the greenhouse were favorable for the photosynthetic metabolism of malic acid, those same temperatures did not benefit the accumulation of reducing sugar during fruit growth.

Conclusion

The antioxidant activity accompanied the increase of phenolics concentration during the first stages of the fruit growth in both treatments. The concentrations of reducing sugars, total phenolic content and acidity increased at the end of the growth in the pineapple fruits. Although there was higher metabolization rate of malic acid in the greenhouse, field cultivation conditions (radiation, temperature) were more favorable for the accumulation of reducing sugars in fruits. However, this increase would not compensate the benefit of the greenhouse cultivation system during low-temperature seasons.

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