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Influence of combined sources of nitrogen fertilization on quality of cv. Vitória pineapple

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Vitória pineapple besides surpassing cultivars traditionally consumed in Brazil, such as Pérola and Smooth Cayenne, exhibits resistance to *Fusarium*. In this sense, nitrogen fertilization is a critical factor because it interferes with the production and postharvest quality regardless of variety. Thus, this research aimed to evaluate the influence of doses and sources of nitrogen on the content of carbohydrates, bioactive compounds and antioxidant activity of Vitória pineapple cultivar. Five doses of N were tested in two sources: Organic (chicken manure) and mineral (urea) using a control with NPK. The lowest doses of N applied (2.62 and 4.50 g/plant) resulted in the contents of total soluble and non-reducing sugars different for the control.Without, the use of 152 g/plant chicken manure resulted in ascorbic acid accumulation. Lower doses of urea resulted in the highest yellow flavonoids, and when combined with chicken manure, it resulted in higher antioxidant activity and ascorbic acid content. Principal components analysis explained 64.7% of variability covering most of the variables analyzed, except for total antioxidant activity by the DPPH$^{\bullet}$ method. All together, the use of chicken manure combined with urea was effective in improving the quality of Vitória pineapple at doses of up to 4.5 g N/plant.

Key words: *Ananascomosus*, chicken manure, nitrogen levels, carbohydrates, bioactive compounds, antioxidant activity.

INTRODUCTION

Pineapple (*Ananascomosus* var. *comosus*) is cultivated in many countries, as it is a fruit of great adaptation in tropical and subtropical regions, with wide acceptance in the consumer market (Crestani et al., 2010). This fruit is of great economic importance to Brazil, which in 2013, ranked as second in world production with 2,483,831.00

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Table 1. Different levels of nitrogen sources (chicken manure - CM and urea) and conventional fertilization (NPK) applied to Vitória pineapple cultivar.

Treatment	Levels		Urea (g/plant)	CM (g/plant)	N (g/plant)
T1	-1	-1	2.91	44.0	2.62
T2	-α	0	0.00	152.0	4.50
T3	0	-α	10.00	0.0	4.50
T4	1	-1	17.10	44.0	9.00
T5	-1	1	2.91	260.0	9.00
T6	0	0	10.00	152.0	9.00
T7	α	0	20.00	152.0	13.50
T8	0	α	10.00	304.0	13.50
T9	1	1	17.10	260.0	15.38
T10	NPK				9.00

Source: Leonardo et al. (2013).

tons (FAOSTAT, 2015). Pérola pineapple cultivar is the most produced in Brazil, especially in the Northern and Northeastern regions, with greater acceptance by Brazilian consumers (Meletti et al., 2011). However, one of the biggest bottlenecks in the production of this cultivar is Fusarium, a disease caused by the fungus *Fusarium subglutinans*, which creates opportunities for the introduction of varieties resistant to this disease such as Vitória cultivar, which in turn, has agronomic characteristics similar to or better than Pérola and Smooth Cayenne cultivars (Ventura et al., 2009).

Besides the selection of varieties, a major factor in the pineapple production chain is fertilization, which makes use of formulations that seek to increase productivity and reach high quality. Higher doses of N, for example, increase productivity, size, and fresh weight of pineapple MD2 (Spironello et al., 2004) and Vitória cultivars (Silva et al., 2012, Caetano et al., 2013). The use of organic materials as a source of N in pineapple crop has been studied to obtain optimum doses of these materials and satisfactory productivity, and also to verify the influence of N on the quality of fruits. In this context, organic sources, alone or combined with mineral sources, have been increasingly used in an attempt to adapt to the trend of establishing sustainable agricultural production using organic raw materials rich in important nutrients (Singh, 2009; Liu and Liu, 2012).

It has been recently reported that the use of chicken manure and urea as sources of nitrogen is effective in increasing the SPAD index and chlorophyll in pineapple leaves (Leonardo et al., 2013), and the use of ammonium sulfate has caused an increase in the chlorophyll content of pineapple leaves of Pérola pineapple (Vieira et al., 2010). In turn, the application of nitrogen on the production of pineapple can also affect aspects of fruit quality, since increases in the doses of N have reduced the titratable acidity and contents of soluble solids and ascorbic acid (Silva et al., 2012; Omotoso and Akinrinde, 2013).

Although it has been reported that organic fertilization using, among other sources, chicken and bovine manure when applied alone, resulted in lower productivity (Mueller et al., 2013), the evaluation of its use combined with other mineral sources can be an alternative to these inputs, which are locally available, reducing production costs. Thus, there is still little information about the influence of organic fertilization on the quality of pineapple, especially the Vitória cultivar, which is still in the introduction phase in Brazil but with great potential for large-scale production. Thus, the aim of this study was to evaluate the influence of chicken manure as source of N, isolated and/or combined with urea, and doses of N on the carbohydrate content, bioactive compounds, and antioxidant activity of Vitória pineapple.

MATERIALS AND METHODS

Experimental conditions

The field experimental phase was conducted at Quandu Farm, Itapororoca municipality, Paraíba State (PB), Brazil. The prevailing climate in the region is As', hot and humid with autumn-winter rains, a drought period of five to six months, and temperatures ranging from 22 to 26°C; the average annual rainfall is 1,500 mm, with variations among the months of cultivation, and higher rainfalls in the months of April, May, and July, with approximately 300 mm (AESA, 2012). Rainfall data were collected in the municipality of Araçagi, PB, located about 9 miles from the municipality of Itapororoca, PB. The experiment used Vitória pineapple (*Ananas comosus* var. Comosus) cultivar.

A randomized block design with four replicates was used. In this experiment, it was usedfive doses of N, according to Silva et al. (2009), in three different application times in two sources, organic (chicken manure) and mineral (urea). This totaled nine combinations determined by central composite matrix of Box (Mateus et al., 2001) + control using NPK (9 g of N/plant; 2.5 g of P_2O_5/plantand 12 g of K_2O/plant) (Table 1). Planting was carried out on February 2010 with seedlings originated from Embrapa Mandioca e Fruticultura, located in the municipality of Cruz das Almas, Bahia State, produced by *in vitro* cultivation. Seedlings were implanted in a double row system with spacing of 0.9 × 0.40 × 0.40

m, density of 38,400 plants/ha. Planting fertilization consisted of 154 kg of P_2O_5/ha of simple superphosphate and topdressing, conducted in three plots. Urea was applied at 2 and 11 months and chicken manure at 2 (in the form of mixture with urea) and 6 months.

Soil had a sandy texture and the following chemical characteristics: pH=5.5; P (melich1)=3.3 mg/dm; K=74.28 mg/dm; Na^+=0.2 $cmol_c$/dm; H+Al=11.22 $cmol_c$/dm; Al=0.2 $cmol_c$/dm; Ca=3.05 $cmol_c$/dm; Mg=1.25 $cmol_c$/dm; SB=4.75 $cmol_c$/dm; CTC=15.97 $cmol_c$/dm; V=29.74%; m=4.04%; M.O.=8.65 g/kg. Chicken manure used in the experiment had the following composition: M.O.=602.2 g/Kg; C=349.2 g/Kg; N=34.5 g/Kg; P=13.7 g/Kg; K=46.5 g/Kg; S=0.3454 g/Kg; Mg=5.5 g/Kg; C/N ratio=10/1. During draught periods, sprinkler irrigation was conducted, which is a practice used in the growing region. Flowering occurred naturally from the second fortnight of August 2011. Harvest was conducted in the second fortnight of December 2011, having passed 22 months from planting to harvest, when most fruits had reached physiological maturity, showing green coloration, with early yellow spots at the base and fruitlets detachment. Evaluations were conducted at the Laboratory of Biologia e Tecnologia Pós-Colheita, Centro de Ciências Agrárias, Universidade Federal da Paraíba (CCA/UFPB), Brazil. An experimental unit consisting of a plot containing 50 plants was used, eliminating the five plants at the extremities, and considering the useful area to be the 40 central plants; 12 fruits were evaluated, randomly harvested from each plot.

Carbohydrates

The contents of total soluble, reducing, and non-reducing sugars (g/100 g) were determined by spectrophotometry (Thermo Scientific, Genesys™ 10S UV-VIS) using dinitrosalicylic acid (DNS) according to Miller (1959). The extract was prepared in the ratio of 2:100 (g pulp:mL distilled water). For the determination of reducing sugars, homogenate was placed in a water bath (60-70 °C) for 5 min, cooled in an ice bath, and filtered. For the determination of total soluble sugars, 2 ml of HCl 1:1 (by volume) was added to 25 ml of extract, placed in a water bath (60-70 °C) for 30 min followed by an ice bath and neutralization with NaOH (2 M), completing the final volume to 100 mL with distilled water. Both determinations were performed based on the standard glucose curve (ranging from 0 to 1801.6 µg), given the linearity range of the spectrophotometric reading of samples centered on the curve using 1.5 mL of extract + 1 mL of DNS reagent. Subsequently, tubes were placed in a water bath at 100°C for 5 min using an ice bath to stop the reaction. For the control sample (blank), the same procedure was performed, replacing the extract volume by distilled water. The final volume of the tube was completed with 10 mL distilled water. The absorbance reading was performed in a spectrophotometer at 540 nm (Genesys™ 10S UV-VIS).

Bioactive compounds

The ascorbic acid content (mg/100 g) was determined by titration using a DPI solution (2,6-dichloro-phenol-indophenol 0.002%) until a permanent light pink coloration was obtained, according to AOAC (2005). The total chlorophyll content present in the peel (mg/100 g) was determined by spectrophotometry (Genesys™ 10S UV-VIS Spectrophotometer) at 652 nm as recommended by Bruinsma (1963), using 2 g of pineapple peel and 10 mL of acetone extraction solution at 80% (w:v); the sample was manually macerated using a mortar.

Yellow flavonoids from the pulp (mg/100 g) were determined according to a method by Francis (1982), using a mixture of 85 mL P.A ethanol and 15 mL of 1.5 M HCl solution for the extraction.

Then, centrifugation was performed at 9000 rpm for 5 min, removing the supernatant and completing the final volume to 15 mL with the extraction solution. Spectrophotometric reading at 374 nm was carried out in quartz cuvettes using the spectrophotometer, Genesys™ 10S UV-VIS.

To determine total extractable polyphenols and antioxidant activity, phenolic extract was obtained according to Dantas et al. (2015). About 5 g of pulp frozen at -85°C was used and added to 4 mL of 50% methanol, left to stand for 1 h for extraction, and centrifuged for 15 min at 15,000 rpm. The supernatant was then removed and placed in a graduated test tube. About 4 mL of 70% acetone was added to the residue and allowed to extract for 1 hour, and centrifuged for 15 min at 15,000 rpm. The supernatant was removed and placed together with the first supernatant, supplementing the volume to 10 mL with distilled water. All procedures were performed in the absence of light.

The quantification of total extractable polyphenols (TEP), expressed in mg of gallic acid/100 g of pulp was performed according to Dantas et al. (2015). Briefly, an aliquot of 0.2 mL of phenolic extract was diluted to 1 mL with distilled water; this aliquot was defined based on the standard curve of gallic acid (0 to 50 mg/µg), considering the absorbance reading based on the linearity range of the standard curve (R^2 =0.99). This solution was added to 1 mL of Folin-Ciocalteau reagent, 2 mL of 20% sodium carbonate, and 2 mL of distilled water, incubated for 30 min in the dark. For the control, the extract volume was replaced by distilled water. Reading was performed in a spectrophotometer (Genesys™ 10S UV-VIS) at a wavelength of 700 nm.

Total antioxidant activity

Total antioxidant activity was determined by capturing $DPPH^•$ (Dantas et al., 2013) and $ABTS^{•+}$ (Dantas et al., 2015) free radicals. For a determination through the DPPH method, three dilutions (200, 600, and 1000 µL/mL) were prepared in triplicate by previous tests based on the standard curve of $DPPH^•$ (final concentration ranging from 0 to 60 µM, diluted in P.A. methanol). From each dilution, an aliquot of 100 µL added to 3.9 mL of $DPPH^•$ radical (60 µM) was used. As a control, 100 µL of the control solution (4 mL 50% methanol; 4 mL 70% acetone; 2 mL distilled water) was used. To calibrate the spectrophotometer (Genesys™ 10S UV-VIS) at a wavelength of 515 nm, P.A. methyl alcohol was used. Results were expressed by the of EC_{50} value, which aims to provide numerical parameters of how much fresh fruit mass is able to provide antioxidants and verify their effectiveness in the scavenging of $DPPH^•$ (g of fruit/g $DPPH^•$).

For total antioxidant activity using the $ABTS^{•+}$ method (µg of $Trolox^•$/g pulp) the radical was prepared, consisting of the mixture of 5 mL of 7 $mMABTS^{•+}$ solution with 88 µL 140 mM potassium persulphate solution resting at room temperature for 16 h in the absence of light. Before testing, the radical was diluted in ethyl alcohol until an absorbance of 0.700 ± 0.05 at 734 nm. The phenolic extract was used to prepare three dilutions of 167, 334 and 501 mg/mL. About 3.0 mL of $ABTS^{•+}$ radical (Abs. 0.700) was added to 30 µL of each dilution and the absorbance was read after 6 min at 734 nm. For determination, the Trolox standard curve with concentrations ranging from 100 to 2000 µM was used, respecting the linearity range of the curve. The results were expressed as µM of $Trolox^•$/g fresh mass. Absorbance readings for these evaluations were performed in a Genesys™ 10S UV-VIS spectrophotometer.

Statistical analyses

Data were submitted to analysis of variance. To evaluate the effect of doses, polynomial regression analysis was used, considering significance up to the quadratic effect for representing the variable

Table 2. Total soluble sugars (TSS), reducing sugars (RS), and non-reducing sugars (NRS) in the pulp of Vitória pineapple fertilized with different doses of chicken manure (CM) and urea compared with conventional fertilization (Control - NPK).

Treatment	Urea	CM	TSS	RS	NRS
	(g/plant)		(g/100 g)		
T1	2.91	44.0	9.64*	4.44	5.20*
T2	0.00	152.0	9.79*	4.50	5.29*
T3	10.00	0.0	7.96*	3.39	4.57*
T4	17.10	44.0	4.38	3.04	1.34
T5	2.91	260.0	6.64	3.89	2.75
T6	10.00	152.0	5.83	2.99	2.85
T7	20.00	152.0	5.21	3.16	2.05
T8	10.00	304.0	6.29	4.14	2.15
T9	17.10	260.0	5.36	3.28	2.07
T10	NPK		5.13	3.94	1.19

*Values are significantly different from the control (T10 – NPK) according to Dunnett's test (p<0.05).

analyzed with determination coefficient R^2>0.60. The means of treatments compared to the control (NPK) were compared by the Dunnett test at P <0.05 error probability. Principal component analysis (PCA) was performed as well as cluster analysis to group treatments that showed similarity in relation to the characteristics under study, using SAS 9.3 (2011) software.

RESULTS

Carbohydrates

When the combination of urea and chicken manure was used in the fertilization of Vitória pineapple cultivar, significant differences were observed between contents of total soluble and non-reducing sugars for the treatments T1, T2, and T3, compared to conventional fertilization (control-NPK) (Table 2). In the absence of urea and using chicken manure (T2: Urea=0 and chicken manure=152 g/plant), an increase in the contents of total soluble and non-reducing sugars was observed, which can be attributed to the high levels of K present in chicken manure. However, the same effect was observed when 10 g/plant of urea (T3) was used alone, with no difference from the other combinations. In relation to reducing sugars, no influence of the different sources of nitrogen (urea and chicken manure) in relation to the use of NPK was observed, reflecting its greater influence on the accumulation of sucrose.

The content of reducing sugars (RS) in Vitória pineapple showed a linear decrease from 4.5 to 3.2 g/100 g glucose with increasing doses of N, which was probably due to the increasing doses of urea. However, the influence of doses of chicken manure or the interaction between the two sources on the content of RS was not verified (Figure 1A), indicating that chicken manure can be applied in the fertilization of Vitória pineapple even at high levels of N without affecting the contents of RS. The effect of doses of urea and chicken manure and the interaction between these two sources of nitrogen on the contents of total soluble and non-reducing sugars was observed (Figure 1B and C). The simultaneous increase of both sources of nitrogen decreased the contents of these sugars. However, it was observed that smaller doses of N provided greater content of sugars. For non-reducing sugars, lower contents (1.05 g/100 g) were found with the application of maximum doses of 304.00 and 15.85 g/plant of chicken manure and urea, respectively.

Bioactive compounds

Sources of nitrogen influenced the contents of ascorbic acid, yellow flavonoids, and total antioxidant activity of Vitória pineapple in relation to fertilization with NPK. However, for the content of total extractable polyphenols (TEP) and total chlorophyll in the peel, no difference for the kind of fertilization was observed, with values ranging from 16 to 23 mg EAG/100 g for TEP and from 3.36 to 4.36 mg/100 g for chlorophyll (Table 3).

The content of yellow flavonoids was higher when plants were fertilized using a combination of 10 g/plant urea and 152 g/plant chicken manure (T6), as compared to control fruits. A mean flavonoids content ranging from 0.19 to 0.52 mg/100 g fresh weight was observed (Table 3), decreasing with increasing doses of urea and chicken manure alone (Figure 2A and B). However, this decrease occurred from 10 g/plant in relation to urea (Figure 2A), and to chicken manure from 152 g/plant (Figure 2B).

Regarding the content of total chlorophyll in the peel of Vitória pineapple, the influence of the interaction between the two sources of N was observed, showing an increase with increasing levels of N (Figure 2C). From the model obtained by the response surface, the combination that resulted in higher total chlorophyll content was 272.01 g/plant chicken manure and 15.85 g/plant urea.

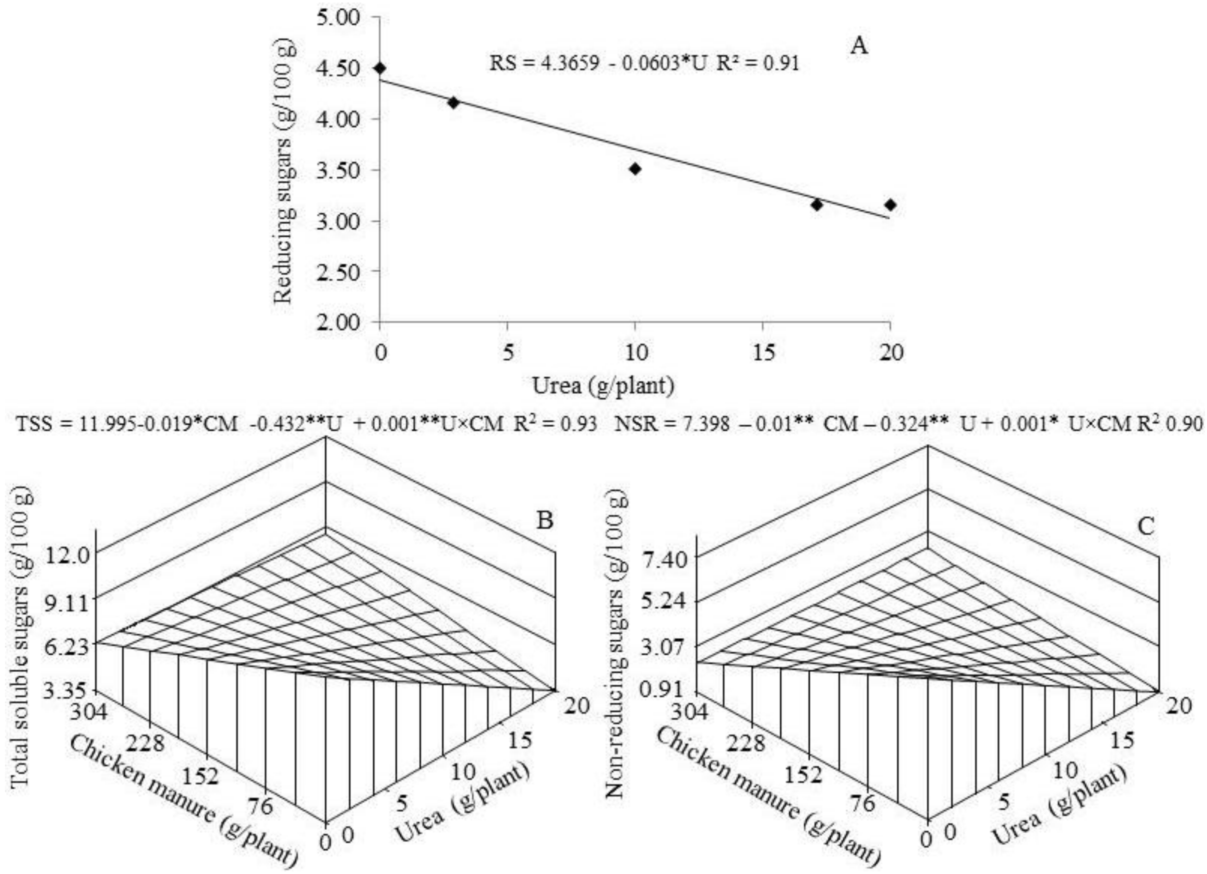

Figure 1. Isolated effect of urea doses on the reducing sugars (RS) (A), and combined effect of chicken manure and urea doses on the total soluble sugars (TSS) (B) and non-reducing sugars (NRS) (C) contents in the pulp of the Vitória pineapple. * Significant t test ($p < 0.05$). ** Significant t test ($p < 0.01$).

Table 3. Bioactive compounds (mg/100 g FW) and total antioxidant activity ($ABTS^{•+}$ - uMTrolox/g pulp, DDPH – g pulp/$DPPH^{•}$ g in the pulp of Vitória pineapple fertilized with different doses of chicken manure (CM) and urea compared with conventional fertilization (Control - NPK).

Treatment	Urea	CM	Ascorbic acid	Yellow flavonoids	Chlorophyll	TEP	$ABTS^{•+}$	$DPPH^{•}$
	(g/plant)							
T1	2.91	44.0	14.08	0.36	3.52	19.98	1.79*	7,466.07
T2	0.00	152.0	18.36	0.34	3.92	16.55	1.41	7,483.58
T3	10.00	0.0	13.69	0.41	4.31	19.14	1.62	5,565.72*
T4	17.10	44.0	10.81*	0.33	3.48	18.72	1.51	5,512.69*
T5	2.91	260.0	8.31*	0.35	3.41	17.04	1.25	7,322.00
T6	10.00	152.0	9.83*	0.52*	4.36	18.72	1.47	8,003.71
T7	20.00	152.0	9.50*	0.30	3.60	23.33	1.63	6,587.53
T8	10.00	304.0	16.19	0.29	3.36	18.13	1.47	7,975.43
T9	17.10	260.0	15.73	0.19	3.67	21.01	1.70	6,446.10
10	NPK		17.60	0.30	3.42	17.60	1.10	8,668.26

*Values are significantly different from the control (T10 – NPK) according to Dunnett's test ($p<0.05$). TEP = total extractable polyphenols.

The ascorbic acid content declined when plants were fertilized with the combination of the following doses: 17.1 and 44 (T4); 2.91 and 260 (T5); 10 and 152 (T6); and 20 and 152 g/plant (T7) of urea and chicken manure,

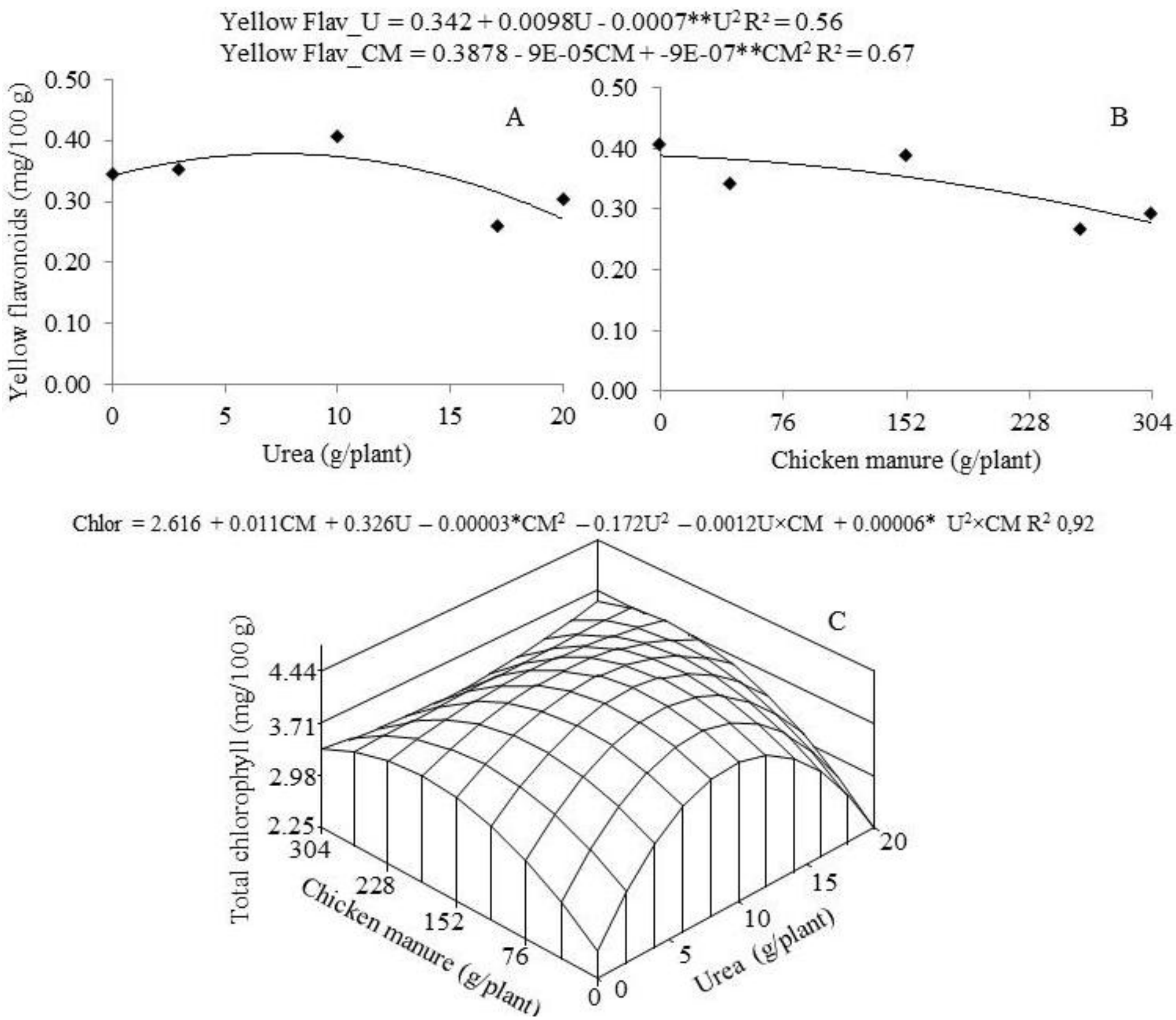

Figure 2. Isolated effect of chicken manure and urea doses on the yellow flavonoid content of the pulp (A and B) and combined effect (C) on chlorophyll content of the peel in Vitória pineapple. * Significant t test ($p < 0.05$). ** Significant t test ($p < 0.01$).

respectively, when compared to control (NPK). In the absence of urea, the use of chicken manure (T2) resulted in accumulation of ascorbic acid, showing 18.36 mg/100 gas the highest mean ascorbic acid content (Table 3), although not significantly different from control. Also, in relation to the ascorbic acid content, an adjustment to the response surface model was observed (Figure 3A), and the combination of urea and chicken manure resulted in maximum accumulation of this bioactive compound at doses of 7.89 and 17.66 g/plant, respectively.

Total antioxidant activity

In relation to total antioxidant activity (TAA) by the $ABTS^{\bullet+}$ method, difference from the control was observed for T1 only (2.91 g/plant urea and 44 g/plant chicken manure), which showed higher antioxidant activity, whose combination was equivalent to 2.62 g N/plant (Table 3). Also, for the TAA by the $ABTS^{\bullet+}$ method, the influence from the interaction of both sources of N was observed (Figure 3B), and the TAA was higher at the maximum doses of urea and chicken manure.

In relation to total antioxidant activity by the $DPPH^{\bullet}$ method, when plants were fertilized with 10 g/plant of urea alone (T3) and the combination of 17.1 g/plant urea and 44 g/plant chicken manure (T4), higher activity compared to control was verified for the fruits (Table 3), since plants submitted to these treatments showed greater ability to scavenge the $DPPH^{\bullet}$ radical, and

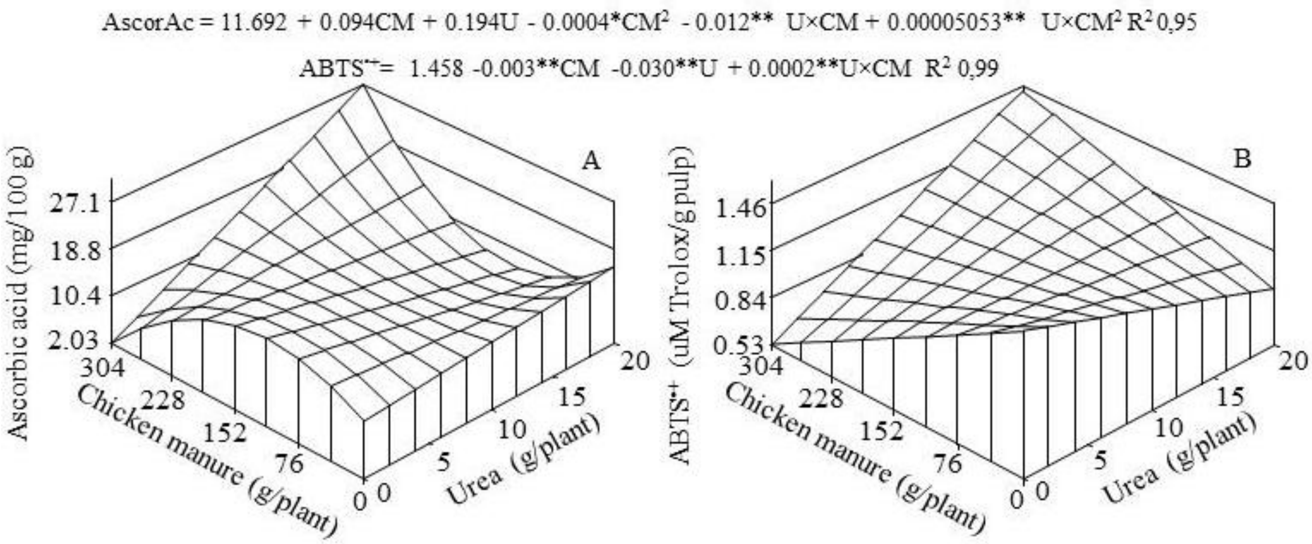

Figure 3. Effect of chicken manure and urea doses combinations on the ascorbic acid content (A) and total antioxidant activity (B) in the pulp of the Vitória pineapple. * Significant t test ($p < 0.05$). ** Significant t test ($p < 0.01$).

Table 4. Principal components (PC1 and PC2) and cumulative variance (CVA) for carbohydrates, bioactive compounds and total antioxidant activity of the 'Victory' pineapple fertilized with urea, chicken manure (CM), and NPK doses.

Variables	Eigenvectors	
	Compound 1	Compound 2
Total soluble sugars	0.478*	0.290
Reducing sugars	0.504*	-0.163
Non-reducing sugars	0.405*	0.418*
Ascorbic acid	0.351*	-0.182
Flavonoids	0.102	0.339*
Chlorophyll	0.041	0.473*
Total extractable polyphenols	-0.364*	0.209
$ABTS^{\bullet +}$	-0.112	0.438*
$DPPH^{\bullet}$	0.283	-0.328
Eigenvalue	3.142	2.539
Cumulative variance (%)	34.91	63.12

*, Indicate the variable correlation with the principal component according to the selection technique of Jolliffe (1972).

therefore, a smaller amount of pulp was required.

Principal components and cluster analysis

Table 4 shows the scores; Figure 4 shows the circle of eigenvectors of variables in relation to the characteristics of quality, bioactive compounds and antioxidant activity of Vitória pineapple fertilized with different levels of N, using urea and chicken manure as sources of N. To satisfactorily explain the variability between treatments, two principal components (PC) were used, with cumulative variance of 63.12%, which was composed of 34.91% for PC1 and 28.21% for PC2. The variables related to principal components were established based on the eigenvectors using the criterion of Jolliffe (1972). For PC1, the main variables that explained the variability between treatments were total soluble, reducing, and non-reducing sugars, and total extractable polyphenols, while non-reducing sugars, yellow flavonoids, chlorophyll, and antioxidant activity by the $ABTS^{\bullet +}$ method were correlated with PC2. No correlations with major

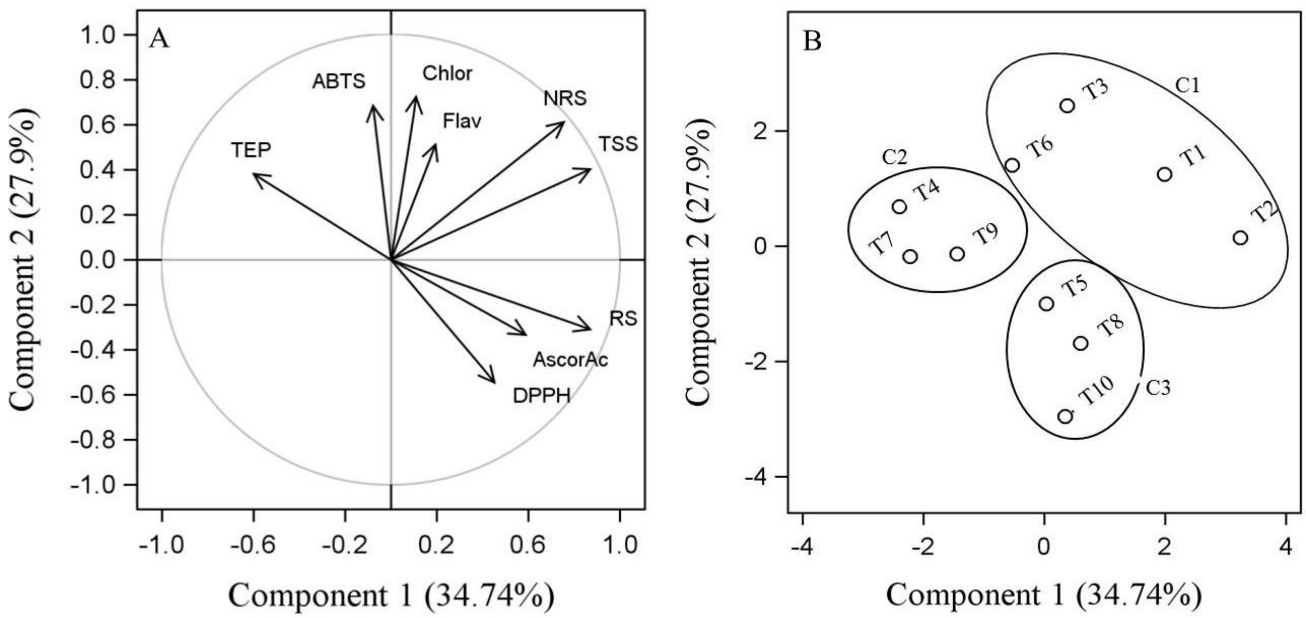

Figure 4. Eigenvectors circle (A) for the variables total soluble sugars (TSS), reducing sugars (RS), non-reducing sugars (NRS), ascorbic acid (AscorAc), yellow flavonoids (Flav), chlorophyll (Chlor), total extrable phenolics (TEP) and total antioxidant activity for ABTS•+ and DPPH•, and clustering of treatments, T1 – T10 (B) of Vitória pineapple based on principal components scores

components related to the content of ascorbic acid and total antioxidant activity by the DPPH• method were verified, since these showed the lowest scores. Thus, these variables do not appear to be important in explaining the variability of treatments used in this work.

From similarities observed in the characteristics of Vitória pineapple, the treatments applied (sources and doses of N) were separated into three clusters (Figure 4B). Treatments T1, T2, T3, and T6 formed the first cluster (C1). The second cluster (C2) was formed by T4, T7, and T9, and the third cluster (C3) comprised treatments T5, T8, and T10.

DISCUSSION

In a study with different pineapple hybrids resistant to *Fusarium*, the fertilization system was not mentioned; Viana et al. (2013) reported content of total soluble sugars (TSS) around 10.0 g/100 g and reducing sugars (RS) around 3.0 g/100 g for Vitória pineapple. The type of field management also affects the sugar contents in pineapple. In Pérola pineapple, Martins et al. (2012) reported that the contents of reducing and non-reducing sugars were delayed during storage in fruits from the integrated production system, compared with those from the conventional. However, it was observed that the contents of sugars for Pérola and Vitória pineapples were similar (Table 2 and Figure 1A and C), which was influenced by the source and levels of nitrogen in relation to conventional fertilization (NPK).

It is noteworthy, however, that the application of higher doses of N increases leaf mass and reduces the partition of photoassimilates of the fruit. This is due to the consumption of carbon skeleton of photosynthesis and energy, in the form of ATP and NADPH, in the nitrogen assimilation. Glutamine synthetase carries out the assimilation of ammonia into glutamine in an ATP-dependent reaction, and nitrate reductase reduces nitrate into nitrite in a key reaction in the nitrogen assimilation by plants (Foyer et al., 2011;Xu et al., 2012).

For total soluble sugars (TSS), Isuwan (2014) observed no effect from using different doses of chicken manure (0-9 g N/plant) on the levels of Pattavia pineapple cultivated in Thailand. However, in this study, it was observed that the decrease in the content of sugars occurred from the dose of 9 g N/plant (Table 2 and Figure 1B). Moreover, Liu and Liu (2012) reported for Cayenne pineapple that the use of organic fertilization compared to conventional increased the content of TSS. Here, it was observed that the use of chicken manure at low levels of N increased the content of sugars in Vitória pineapple. Therefore, it was clear that at low levels of N (2.62 and 4.50 g/plant), compared with urea, chicken manure meets the demand of other nutrients such as K, for growing pineapple (Figure 1).

It has been reported that nitrogen affects the quality aspects of pineapple. In this context, the content of soluble solids decreases due to increased levels of N (Caetano et al., 2013;Omotoso and Akinrinde, 2013), and this reduction is related to the increase of fruit massin the presence of higher amounts of N, which can cause dilution of compounds (Teixeira et al., 2002). However, for Cayenne pineapple grown in China, an increase in the content of non-reducing sugar with the use of organic fertilizer was observed (Liu and Liu, 2012).

In a study with fertilization of Smooth Cayenne pineapple, Ma et al. (2013) reported that different N-P-K ratios did not significantly influence the content of soluble sugars. However, the content of ascorbic acid increased by approximately 30% with fixed doses of N (400 kg/ha) and P (100 kg/ha), varying the application of K from 0 to 800 kg/ha. Titratable acidity, in turn, decreased by 17% when there was variation in the supply of N (0-600 kg/ha), and increased by almost 24% when only potassium was applied in increasing doses (0–800 kg/ha). Potassium (K) is of great importance in many processes where the final quality of fruits and vegetables directly or indirectly depends on it. This is because K is a cofactor of photosynthesis enzymes, acting in the translocation of photoassimilates to different plant sinks (Armengaud et al., 2009; Bidari and Hebsur, 2011).

The increase in the content of ascorbic acid with the application of chicken manure occurs is probably due to the supply of K, which thus promotes an increase in the biosynthesis of photoassimilates that will lead to increased production of precursors for the synthesis of this acid (Lester et al., 2010). Viana et al. (2013) reported ascorbic acid content around 16 mg/100 g for Vitória, 21 mg/100 g for Pérola, and 15 mg/100 g for Smooth Cayenne. Therefore, the ascorbic acid content of Vitória is similar to Pérola and Smooth Cayenne pineapples. In addition, the production system may affect the ascorbic acid content, since Perola pineapple from a conventional system stored at room conditions of 23± 2°C, 65±5% RH, tended to show a higher content when compared to that from an integrated system (Martins et al., 2012).

Many pre and postharvest factors can influence the ascorbic acid content in fruits and vegetables, including levels of N applied during cultivation (Lee and Kader, 2000). For Smooth Cayenne pineapple, no change was observed in the ascorbic acid content using different doses and sources of nitrogen (Teixeira et al., 2002;Spironello et al., 2004). In turn, Isuwan (2014) reported an increase in the contents of these compounds with increasing doses of chicken manure (0-9 g N/plant) in Pattavia pineapple grown in Thailand, which was explained by the mineral composition of this source of fertilization. On the other hand, a decrease was reported in the ascorbic acid content in pineapple pulp due to increased levels of N by Omotoso and Akinrinde (2013) for pineapple grown in Nigeria. In this context, Ramos et al. (2010) reported that N deficiency promoted increased levels of ascorbic acid in Imperial pineapple grown in a greenhouse in Brazil. This probably occurred because N deficiency reduced the rate of protein biosynthesis, promoting greater availability of photoassimilates to be used in the synthesis of secondary metabolic compounds, such as ascorbic acid that may act in plant defense (Marschner, 2012).

The content of flavonoids found for Vitória pineapple in this work, regardless of treatment, was similar to that reported by Hossain and Rahman (2011) for pineapple cultivar grown in India, and is lower than that reported by Alothman et al. (2009) in pineapple grown in Malaysia using different extraction solutions, both in cultivation and fertilization systems not informed. In tomato and lettuce, Stefanelli et al. (2010) emphasized that the application of lower doses of N resulted in higher content of flavonoids. According to these authors, under the condition of N deficiency, the phenylalanine ammonia lyase (PAL) tends to increase its activity to meet the demand of N in the cell from phenylalanine, leading to a greater production of phenolic compounds, among them flavonoids.

For the antioxidant activity, in MD-2 pineapple fertilized with different K/N ratios, Silva (2010) reported values ranging from 7,348 to 13,463 g pulp/g DPPH$^{\bullet}$, with the highest activity observed for the ratio of 3:1, which may have been influenced by the increase in doses of K. Here, the highest antioxidant activity was observed when the highest doses of chicken manure alone were applied, which can also be attributed to the resulting increased supply of K of this source of fertilization.

The decrease in the antioxidant activity promoted by the simultaneous increase in doses of urea and chicken manure probably occurred as a consequence of the decrease in the content of bioactive compounds responsible for this activity, such as flavonoids (Figure 2A and B) and ascorbic acid (Figure 3A). In this context, changes in antioxidant activity related to the increase in the supply of N to plants have been reported (Lata, 2014;Zhang et al., 2014). In chrysanthemum (*Chrysanthemum morifolium*), Liu et al. (2010) observed a reduction in antioxidant activity with increased N supply and attributed this reduction to the competition for L-phenylalanine, a precursor of both proteins and polyphenols, since these compounds inhibit the oxidizing action. For *Labisiapumila* (a medicinal herb from southeastern Asia), the activity of phenylalanine ammonia lyase was reduced with increasing doses of N, and it was observed that this behavior was accompanied by a reduction in the content of flavonoids and phenolic compounds (Ibrahim et al., 2011).

In relation to the peel chlorophyll content, although no difference was observed in the use of different sources of nitrogen (urea, chicken manure and NPK) in the fertilization of Vitória pineapple, the effect of the interaction between the sources urea and chicken manure was observed. This effect on the chlorophyll content has been observed for pineapple leaves with the use of different sources of nitrogen fertilization such as humic acids (Baldotto et al., 2009), ammonium sulfate (Vieira et al., 2010), and chicken manure and urea (Leonardo et al., 2013). Silva et al. (2012) reported that the increase in the levels of N increased the content of N in chlorophyllousportions of pineapple leaves, also influencing the composition of this element in relation to other mineral nutrients. Therefore, since the photosynthetic efficiency is directly related to chlorophyll content, N availability can decisively influence the production capacity of plants (Taiz and Zeiger, 2008; Stefanelli et al., 2010).

Cluster (C1) was formed by treatments T1, T2, T3 and T6 for presenting similarities mainly in relation to total soluble, reducing, and non-reducing sugars, showing higher content compared to the other treatments, with the exception of T6, which showed lower content. Thus, T6 was grouped to C1 due to the similarity in total extractable polyphenols, with intermediate content, and in total antioxidant activity by the ABTS$^{\bullet+}$ method, showing higher activity when compared to C2 and C3. The higher content of sugars in T1, T2, and T3 was probably due to the use of lower doses of N (2.62, 4.50 and 4.50 g N/plant, respectively).

Treatments T4, T7, and T9 (C2), which were fertilized with different doses of nitrogen and higher doses of urea, showed lower content of total and reducing sugars as compared to the other clusters. This cluster also showed increased antioxidant activity by the DPPH$^{\bullet}$ method. For the third cluster (T5, T8, and T10), similarities were observed in total antioxidant activity, which was lower than the other clusters, and also in total sugars, where the contents were intermediate to C1 and C2. The similarity between treatments T5 and T8, therefore, may be due to the use, for both treatments, of the combination of higher doses of chicken manure and lower doses of urea.

The combined use of chicken manure and urea as sources of nitrogen at doses of 2.62 and 5.50 g/plant, respectively, in the cultivation of Vitória pineapple was effective in improving characteristics related to flavor (sugar content) and functional properties (ascorbic acid, flavonoids, and antioxidant activity), and the use and doses above 9 g N/plant is not recommended. However, further studies are needed to show the relationship between mineral sources of nitrogen and other nutrients of chicken manure such as potassium, and phosphorus.

Conflict of Interest

The authors have not declared any conflict of interest.

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