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Effect of NPK fertilization on the chemical properties and bioactive compounds of the *cabezona* pineapple fruit

Pérez-Romero Juventino 1 , Salgado-Garcia Sergio \ast1 , Lagunes-Espinoza L. del Carmen 1, Córdova-Sánchez Samuel², Ortiz-García C. Fredy^{*1}

- ¹ Colegio de Postgraduados-Campus Tabasco, Producción Agroalimentaria en el Trópico. Km. 3.5 Periférico Carlos A. Molina S/N. H. Cárdenas, Tabasco. CP 86500. México.
- ² Universidad Popular de la Chontalpa. División Académica de Ciencias Básicas e Ingenierías. CA-QVyDS. Carretera Cárdenas - Huimanguillo, Km. 2.0 Cárdenas, Tabasco, México. CP. 86500.
- * Correspondence: salgados@colpos.mx; cfortiz@colpos.mx

ABSTRACT

Objective: To evaluate the effect of NPK fertilization on the chemical properties and bioactive compounds of the cabezona pineapple fruit.

Design/methodology/approach: 15 NPK fertilization treatments were evaluated and carried out in the field in a random complete block design with four repetitions per treatment. At the end of the crop cycle, we determined the pH, °Brix and citric acid % of the fruits that were harvested, following the methodology established in NMX-FF-028 and 011. The pH of the juice was measured directly using a potentiometer. Ascorbic acid was measured with a SIGMA-ALDRICH® kit, the total polyphenol contents were determined following the methodology of De la Cruz-Ricardez *et al*. (2020), while total flavonoid contents were measured following a modified version of the methodology proposed by Hossain *et al*. (2011).

Results: The mean values for °Brix, citric acid %, and pH were 7.3, 7.1 and 3.4 respectively, without significant differences between treatments. A highly significant difference in the concentration of ascorbic acid was observed between treatments; treatment three had the highest concentration (19.4 mg 100 g^{-1} FF). There were no significant differences between the fertilization treatments and the control, regarding total polyphenol content (41.34 mg 100 g^{-1} FF) and flavonoids (1.6 mg 100 g^{-1} FF) concentrations.

Study limitations/implications: The ripening degree of the pineapple fruit directly influences the chemical properties and the concentration of bioactive compounds.

Findings/conclusions: NPK fertilization had no effect on °Brix and pH. The citric acid percentage and the total polyphenol content increased as the doses of P_2O_5 increased. The ascorbic acid concentration and total flavonoid content are not affected by the NPK fertilization dose.

Keywords: Cabezona pineapple, NPK fertilization, chemical properties, bioactive compounds.

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INTRODUCTION

Pineapple is a source of sugars, organic acids, and some essential minerals for human nutrition (Paull and Chen, 2003); it is also an antioxidant-rich fruit that promotes health; these natural antioxidants include ascorbic acid and phenolic compounds (Hossain *et al*., 2011; Lu *et al*., 2014). Ascorbic acid has a high bioavailability and can inhibit the development of cardiovascular diseases and certain types of cancer, protecting the membranes and the lipoproteins from oxidative damage (Gardner *et al*., 2000; Ferreira *et al*., 2016). The phenolic compounds are responsible for the sourness, astringency, taste, color, and oxidative stability of fruits and vegetables; these compounds protect health, removing free radicals and inhibiting hydrolytic and oxidating enzymes, and they also have antiinflammatory functions (Naczk and Shahidi, 2004). The *Cabezona* pineapple is produced and commercialized in La Chontalpa, Tabasco. However, there is no information about its chemical properties and the bioactive compounds content of its fruit.

MATERIALS AND METHODS

Location

The experiment was carried out in a field where Cayenne, MD2, and *Cabezona* pineapples have been grown for many years. This field is located in the Huimanguillo municipality, Tabasco, at 17° 40" N and 93° 38" W, at 17 masl.

Vegetable Material

Basal shoots (also known as spikes or thorns) from pineapple vegetable material were used for this experiment; these basal shoots came from a *Cabezona* pineapple cultivar and weighted 370 g.

Treatments and Experimental Design

The following NPK levels were evaluated: N (urea) (120, 160, 200, and 240 kg ha⁻¹); P (DAP) (70, 110, 150, and 190 kg ha⁻¹ of P₂O₅); and K (KCl) (150, 200, 250, and 300 kg ha $^{-1}$ of K₂O). Treatments were generated through the Plan Puebla 1 matrix (Turrent, 1985) and were evaluated in a random complete block design with four repetitions.

Study Variables

Fruits with optimal physiological ripeness that had undergone a fertilization treatment were used to determine the following chemical properties: pH, °Brix, and titratable acidity. The following bioactive compounds were determined: ascorbic acid and total polyphenol and flavonoid contents. The pH was directly measured from the juice, using a PC18 Conductronic potentiometer. °Brix were measured directly from the juice, as established in NMX-FF-015-1982, using an Atago® handheld refractometer, with a 0-33 °Brix measurement range. Titratable acidity was determined following the methodology established in NMX-FF-011-1982; the results were expressed as citric acid percentage (%). The ascorbic acid concentration was determined using a SIGMA-ALDRICH® ascorbic acid detection kit; the results were expressed as mg of ascorbic acid 100 g^{-1} of fresh fruit (FF). The total polyphenol content concentration was determined following the method

described by De la Cruz-Ricardez *et al*. (2020) with some modifications. The galic acid was used as standard; the results were expressed as mg $100\,\mathrm{g}^{-1}$ of fresh fruit. The total flavonoid content concentration was determined following a modified version of the methodology described by Hossain *et al*. (2011), using quercetin as standard; the results were expressed as mg $100 g^{-1}$ of fresh fruit.

Statistical Analysis

In order to verify the assumption of normality and homogeneity of the variances, a RDBA ANOVA and a 0.05 Tukey test were carried out to analyze all the variables, using the Rstudio statistical kit, version 3.5.1 (Gentleman and Ihaka, 1993).

RESULTS AND DISCUSSION

Chemical Properties

pH

There was no statistical difference between treatments; in a $\frac{1}{4}$ color (NMX-FF-028-SCFI-2008), the *Cabezona* pineapple had a mean pH of 3.4 (Table 1). This pH value is similar to the results reported by Lu *et al*. (2014) for the Smooth Cayenne #1 (3.58), Smooth Cayenne #2 (3.86), Comte de Paris (3.93), and Ripley (3.91) cultivars. This pH value is also similar to the findings of Da Silva *et al*. (2014): Victoria (3.6), Perola (3.8), Gold (3.8), and EC-93 (3.8) pineapples. In their study, Morales *et al*. (2001) reported 3.8-4.10 pH values for the Indian pineapple.

Figure 1 shows the graphic response to NPK fertilization; in the N-110-200 curve the highest pH value was obtained with 200 kg ha⁻¹ of N, while in the N-150-250 curve the highest pH values were obtained with 160 and 240 kg ha⁻¹. Meanwhile, the highest pH values were obtained with 70, 110, and 190 kg ha⁻¹ of P. Finally, the highest values for the K doses were obtained with 200 kg ha⁻¹. The T6(200-110-250) has an acceptable pH and it was also associated with a higher fresh fruit yield (Pérez-Romero *et al*., 2020).

°Brix

There were no statistical differences on the NPK fertilization regarding °Brix; the mean value was 7.3 °Brix (Table 1). According to the NMX-FF-028-SCFI-2008, in order to be commercialized, MD2, Cayenne Lisa, and Champala pineapple cultivars must have at

Figure 1. Response of the *Cabezona* pineapple juice's pH to NPK fertilization.

Fertilization treatments (kg ha ⁻¹ de N, P ₂ O ₅ , K ₂ O)	pH	°Brix	Citric acid, %
$T1_{(160-110-200)}$	3.4a	7.1a	0.77a
$T2_{(160-110-250)}$	3.4a	7.4a	0.76a
$T3_{(160-150-200)}$	3.4a	7.2a	0.78a
$T4_{(160-150-250)}$	3.5a	7.0a	0.71a
$T5_{(200-110-200)}$	3.5a	6.9a	0.69a
$T6_{(200-110-250)}$	3.5a	7.7a	0.71a
$T7_{(200-150-200)}$	3.5a	7.1a	0.67a
$T8_{(200-150-250)}$	3.4a	7.9a	0.76a
$T9_{(120-110-200)}$	3.4a	7.4a	0.74a
$T10_{(240-150-250)}$	3.5a	7.3a	0.75a
$T11_{(160-70-200)}$	3.5a	7.7a	0.60a
$T12_{(200-190-250)}$	3.5a	5.8a	0.68a
$T13_{(160-110-150)}$	3.4a	7.4a	0.76a
$T14_{(200-150-300)}$	3.4a	7.8a	0.79a
$T15_{(00-00-00)}$	3.4a	6.0a	0.61a
Media	3.4	7.3	0.71
C.V. $(%)$	$0.02\,$	$0.08\,$	$0.08\,$
Prob. De F.	0.28 ^{ns}	$0.18^{\rm ns}$	0.20 ^{ns}
DMS	0.24	2.54	0.27

Table 1. Chemical properties (pH, °Brix, and citric acid %) resulting from the different fertilization treatments carried out in *Cabezona* pineapples.

*Means with the same letters are not statistically different (Tukey, 0.05). C.V.=variance coefficient; DMS=significative minimum difference; Prob. De F.=Fisher probability.

least 12, 11, and 11°Brix respectively, regardless of their ripening degree. This norm does not take into account the *Cabezona* pineapple, probably because it is a cultivar of regional importance. Additionally, the mean value (°Brix) of *Cabezona* pineapples is far below the results reported by other authors, such as Ulloa *et al*. (2015) for Comte de Paris (16.94), Smooth Cayenne #2 (14.95), and Queensland (17.80) cultivars or Da Silva *et al*. (2014) for Victoria (16) and Perola (13.10) cultivars. A possible cause of these low PFix values in the *Cabezona* pineapple could be the ripening degree and the harvest season, because those values increase as the fruit ripens (Rosas *et al*., 2011) and they decrease during the season of North's winds (Uriza *et al*., 2018).

Figure 2 shows, on the one hand, the response of °Brix to the dose of N: when low amounts of fertilization (N-110-200 curve) are used, the °Brix decrease as the doses of N increase. On the other hand, when high fertilization doses (N-150-250 curve) are used, \textdegree Brix increase, reaching their maximum level at 200 kg ha $^{-1}$ of N and then they tend to decrease. Figure 2 also shows the °Brix response to the application of P, where both curves (200-P₂O₅-250 and 160-P₂O₅-200) indicate that ^oBrix decrease as the doses of P increase. Regarding K, there was little difference in ${}^{\circ}$ Brix in the 160-110-K₂O curve when its dose increased. However, when high doses of fertilization $(200-150\text{-K}_2\text{O})$ were applied, \textdegree Brix increased as the doses of K increased. This behavior has already been reported by Py *et*

Figure 2. Response of the pineapple cultivar's ^oBrix to NPK fertilization.

al. (1987), Rebolledo *et al*. (2011), and Uriza *et al*. (2018), who point out that K is directly responsible for the quality of the pineapple fruit.

Citric Acid Percentage

There was no statistical difference between NPK fertilization treatments; the citric acid mean value was 0.73% (Table 1). This value was similar to the results reported by Lu *et al*. (2014) for the Comte de Paris pineapple (0.73%) and the Giant Kew pineapple (0.69%). Additionally, this value falls within the range reported by Rosas *et al*. (2011) for the Esmeralda pineapple (0.4-1.2%). However, this value could increase just like in the case of the Esmeralda pineapple, where the citric acid concentration increases as it ripens (Rosas *et al*., 2011), contrary to what happens with other tropical fruits such as mango, passion fruit, and papaya (Torres *et al*., 2013). This study corroborated that citric acid concentration increases during the rainy season and with the use of KCI as source of the K2O; fertilization treatments exceed the unfertilized control in one unit (Rebolledo *et al*., 2011; Uriza *et al*., 2018).

Citric acid concentration did not show a clear response to the application of high or low doses of N (Figure 3); the highest concentrations in the N-110-200 and N-150-250 curves were obtained with 160 kg ha⁻¹ of N and 200 and 250 kg ha⁻¹ of N, respectively. Figure 3 shows that citric acid concentration has a positive response to the increase of P_2O_5 doses; in both curves (160-P₂O₅-200 and 200-P₂O₅-250), the highest concentration was obtained with 150 kg ha⁻¹ of P₂O₅; concentrations tend to decrease after this dose. Additionally, high doses of $K(200-150-K₂O$ curve) can affect the citric acid concentration; concentration increased as the doses changed from 200 to 300 kg ha⁻¹ (Figure 3).

Bioactive Compounds

Ascorbic Acid Concentration

Highly significant differences were observed in the concentration of ascorbic acid in the NPK fertilization treatments (Table 2). The Tukey Test establishes five groups: the T13 and T15 treatments obtained the lowest concentration, while the T3, T6, T8, and T10 treatments obtained the highest concentrations. These results match those reported by Samson (1991), who found out that the ascorbic acid of pineapples varies from 8 to 30 mg 100 g^{-1} FF. Several authors have observed wider intervals (5.08-62.11 mg 100 g^{-1} FF)

Figure 3. Response of the *Cabezona* pineapple juice's citric acid percentage to NPK fertilization.

<i>Cabezona</i> pineapple.						
Fertilization treatments	Ascorbic acid	Total polyphenols	Total flavonoids			
(kg ha ⁻¹ de N, P ₂ O ₅ , K ₂ O)	$(mg 100 g-1$ of fresh fruit)					
$T1_{(160-110-200)}$	14.6abc	41.4a	1.8a			
$T2_{(160-110-250)}$	14.9abc	46.3a	1.5a			
$T3_{(160-150-200)}$	19.4a	43.9a	1.5a			
$T4_{(160-150-250)}$	14.6abc	35.5a	1.4a			
$T5_{(200-110-200)}$	11.8abc	49.3a	1.6a			
$T6_{(200-110-250)}$	19.4ab	40.5a	1.4a			
$T7_{(200-150-200)}$	11.3abc	35.7a	1.2a			
$T8_{(200-150-250)}$	16.0ab	43.8a	1.6a			
$T9_{(120-110-200)}$	14.7abc	41.1a	1.7a			
$T10_{(240-150-250)}$	15.1ab	36.2a	1.6a			
$T11_{(160-70-200)}$	13.4abc	35.7a	1.9a			
$T12_{(200-190-250)}$	11.3abc	42.2a	1.6a			
$T13_{(160-110-150)}$	6.2c	43.2a	1.9a			
$T14_{(200-150-300)}$	11.8abc	42.7a	1.7a			
$T15_{(0-0-0)}$	10.5 _{bc}	42.3a	1.5			
Media	13.67	41.34	1.6			
C.V.	0.25	0.10	0.11			
Prob. De F.	0.001^{**}	$0.30^{\rm ns}$	0.30 ^{ns}			
DMS	8.83	14.28	0.82			

Table 2. Bioactive compounds - ascorbic acid concentration in the different fertilization treatments of *Cabezona* pineapple.

*Means with the same letters are not statistically different (Tukey, 0.05). C.V.=variance coefficient; DMS=significative mean difference; Prob. De F.=Fisher probability.

for various pineapple cultivars, including: Ferreira *et al*. (2016), who reported 35.88- 62.11 mg $100~{\rm g}^{-1}$ FF concentrations; Lu *et al.* (2014) who reported 5.08-33.57 mg $100~{\rm g}^{-1}$ FF concentrations; and Da Silva *et al.* (2014), who reported 7.03-25.48 mg 100 g^{-1} FF concentrations. We must mention that Morales *et al*. (2001) and Rosas *et al*. (2011) reported that ascorbic acid concentration increases in the Esmeralda and Indian pineapples as their fruit ripens. This effect could provide a possible explanation for the lack of much similarity in the concentrations reported by the authors.

Ascorbic acid concentration does not show a clear response when the N doses increases (N-150-250 curve), although the highest concentration was obtained with 250 kg ha⁻¹ of N per hectare (Figure 4); however, as the doses of N increase, the ascorbic acid concentration shows a clear decrease in the N-100-200 curve. With low fertilization doses (160-P₂O₅-200 curve), the ascorbic acid concentration increases as the dose of P increases, reaching a maximum concentration of P at 150 kg ha $^{-1}$. The opposite result is obtained with high fertilization doses $(200-P_2O_5-250)$: the ascorbic acid concentration decreases as the doses of P increase, reaching a minimum concentration of 190 kg ha $^{-1}$. As a result of the positive interaction, the 200-150-K₂O curve, with 250 kg ha⁻¹ of $K₂O$, has the highest ascorbic acid concentration (Figure 4); meanwhile, the ascorbic acid concentration increases in the 160-110-K₂O curve, with 200 and 250 kg ha⁻¹ doses of K_2O , although this increase is lower than the one reported for the 200-150- $K₂O$ curve.

Total polyphenols content

There were no significative differences between fertilization treatments (Table 2). Total polyphenols content had a mean value of 41.34 mg 100 $\rm g^{-1}$ FF. This value is closer to the results reported by Lu *et al.* (2014) for various cultivars (37.48-77.55 mg 100 g^{-1} FF), by Rosas *et al.* (2011) for Esmeralda pineapples (44.78 mg 100 g^{-1} FF), and by Ferreira *et* al. (2016), likewise for several cultivars (71.08-126.95 mg 100 g^{-1} FF). These differences can be the result of the type of cultivar and the ripening degree of the pineapple fruits. Polyphenols content —as well as ascorbic acid content— increases in Esmeralda pineapples as the fruit ripens.

The maximum polyphenols content was found at 200 kg ha⁻¹ of N for high and low fertilization doses (N-110-200 and N-150-250 curve); however, polyphenols content shows a drastic decrease with a 200 to 240 kg ha^{-1} of N increase and high fertilization doses (Figure 5). Increasing doses of P provide a better response: polyphenols content increases as the doses increase, reaching a maximum content with 150 ha^{-1} of P; however, content tends to decrease with higher doses (Figure 5). Meanwhile, potassium had a poor response: the highest polyphenols content was obtained with 250 kg ha⁻¹ of K_2O for both curves (160-110-K₂O and 200-150-K₂O).

Figure 4. Response of the *Cabezona* pineapple juice's ascorbic acid percentage to NPK fertilization.

Figure 5. Response of the total polyphenols content in *Cabezona* pineapples to NPK fertilization.

Total flavonoids content

There were no statistical differences between fertilization treatments regarding the content of total flavonoids content (Table 2). The mean concentration was 1.6 mg 100 g⁻¹ FF.

The flavonoids content in *Cabezona* pineapples were different from the results obtained by Lu *et al.* (2014) —who reported 27.3, 34.5, 17.24, and 19.64 mg 100 g^{-1} FF for MD2, Comte de Paris, Fresh Premium, and Pearl, respectively— and by Hossain *et al*. (2011) —who reported 51.1 mg 100 g^{-1} FF for tropical pineapples. These differences are likely the result of the color of the flesh of the *Cabezona* pineapple, which is paler than the flesh coloration percentage of the MD2 pineapple (63-87%); flavonoids are responsible for the color and taste of fruits and vegetables.

The flesh of the *Cabezona* pineapple could also have a lower browning tendency than other cultivars; therefore, its capacity to synthesize the flavonoids that are involved in the prevention of oxidation would be low and this process allows the protection of existing vitamins and enzymes. Another potential cause could be the low levels of glucose —the precursor molecule of the synthesis of flavonoids— in the flesh (Martínez *et al*., 2002; Mandalar *et al*., 2006).

The effect of N doses on flavonoids content is not clear: the contents obtained with low fertilization doses (N-110-200 curve) are higher than the contents obtained with high fertilization doses (N-150-250 curve) (Figure 6). The flavonoids content response to

Figure 6. Response of the flavonoids content in *Cabezona* pineapples to NPK fertilization.

increasing P and K doses are similar. Content tends to decrease with low fertilization doses $(160-P₂O₅-200$ and 160-110-K₂O curve), while it increases with high doses of the same nutrients (200-P₂O₅-250 and 200-150-K₂O curve), reaching maximum P and K levels of 150 and 200 kg ha⁻¹ and 300 kg ha⁻¹, respectively.

CONCLUSIONS

NPK fertilization did not have a significative effect on pH and °Brix. The application of N and K does not affect the citric acid percentage and the total polyphenols content; however, they increase along with the P_2O_5 doses and they reach a maximum concentration at 150 kg ha $^{-1}$. NPK fertilization did not affect the ascorbic acid and flavonoids concentration.

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