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Effect of nitrogen fertilization on the physical and physicochemical characteristics and antioxidant potential of sapodilla (*Manilkara zapota* L. P.Royen) at different stages of development

Efeito da adubação nitrogenada nos caracteres físico, físico-químico e potencial antioxidante do sapoti (*Manilkara zapota* L. P.Royen) em diferentes estádios de desenvolvimento

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Abstract

This study aimed to evaluate the effect of nitrogen fertilization on the physical and physico-chemical characteristics and antioxidant potential of sapodilla at different stages of development. The experiment was installed at the Norfruit farm, located in the rural community of Pau Branco, Mossoró/RN, Brazil. A randomized block with two factorial schemes experimental design, was adopted. The first 5 x 6 with lost parcels, consisting of five doses of nitrogen (N) (0, 300, 600, 900 and 1200 g of N.plant^{-1}) and six stages of fruit development; and the second 2 x 3 consisting of two levels of nitrogen (N) (0; 600 g of N plant^{-1}) and three stages of development, both with five replications. The fruits were labeled with a 10 to 15 mm long mark in the plant. In fact, were harvested and analyzed after 90, 120, 150, 180 and 200 days of its marking and 208 days (fully mature, after 8 days of storage at 25 ± 2 °C and RH 58 ± 5%), respectively. The results showed a nitrogen fertilization at the dose of 600 g of N.plant^{-1}, produced fruit with higher sugar content and fresh weight, reaching greater mass and sugar content, at 200 and 180 days of fruit development, respectively. An increase in nitrogen fertilization, promoted a reduction in the levels of yellow flavonoids and anthocyanins. Bioactive compounds (total polyphenols, vitamin C, flavonoids and anthocyanins) and sapodilla antioxidant activity decreased with the further development of fruit. The antioxidant activity of the sapodilla is more related to the high content of total polyphenols. The knowledge obtained in this study is extremely important for the sapodilla production chain due to information of the nutritional and antioxidant composition of the fruits. This also proves beneficial for the fruit marketing, because nowadays the public seeks foods rich in antioxidants, and the sapodilla is marketed both in natura and processed, in addition to its use in the cosmetics manufacture.

Key words: Bioactive compounds, chemical composition of fruits, mineral nutrition, ripening.

Resumo

Esse trabalho objetivou avaliar o efeito da adubação nitrogenada nos caracteres fisico, fisico-químico e potencial antioxidante do sapoti em diferentes estádios de desenvolvimento. O experimento foi instalado na fazenda Norfruit, localizada na comunidade rural de Pau Branco, município de Mossoró/RN. O delineamento adotado foi em blocos casualizados com dois esquemas fatoriais, o primeiro 5 x 6 com parcelas perdidas, composto por cinco doses de nitrogênio (N) (0; 300; 600; 900 e 1200 g de N planta^{-1}) e seis estádios de desenvolvimento do fruto; e o segundo 2 x 3, composto por duas doses de nitrogênio (N) (0; 600 g de N planta^{-1}) e três estádios de desenvolvimento, ambos com cinco repetições. Os frutos foram marcados com 10 a 15 mm de comprimento na planta; sendo colhidos e avaliados após 90, 120, 150, 180 e 200 dias de sua marcação e 208 dias (completamente maduros, após 8 dias de armazenamento a 25 ± 2 °C e UR 58 ± 5%). Os resultados obtidos permitiram concluir que a adubação nitrogenada na dose de 600 g de N planta^{-1} produziu frutos com maiores teores de açúcares e massa fresca, tendo atingido maior massa aos 200 dias de desenvolvimento do fruto e maiores teores de açúcares aos 180 dias de desenvolvimento. O aumento da adubação nitrogenada promoveu uma redução nos teores de flavonoides amarelos e antocianinas. Os compostos bioativos (polifenóis totais, vitamina C, flavonoides e antocianinas) e a atividade antioxidante do sapoti reduziram com o avanço do desenvolvimento do fruto. A atividade antioxidante do sapoti está mais relacionada com o elevado conteúdo de polifenóis totais. Os conhecimentos obtidos nesse estudo são de extrema importância para a cadeia de produção de sapoti, devido o mesmo trazer informações da composição nutricional e antioxidante dos frutos. Isso também gera benéficos para a comercialização do fruto, pois atualmente a população busca alimentos ricos em antioxidantes, e o sapoti é comercializado tanto *in natura* como processado, além da sua utilização na fabricação de cosméticos.

Palavras-chave: Amadurecimento, composição química dos frutos, compostos bioativos, nutrição mineral.

Introduction

The sapodilla (*Manilkara zapota* L. P.Royen), is an exotic fruit species that finds in northeastern Brazil excellent conditions for its development and production. It is a very profitable crop due to good acceptance of the product by the consumer.

In Brazil, the first cultivar was developed in 1983 (cv. Itapirema-31), followed by cultivar Chocolate in 1999, both established by researchers from Pernambuco Agricultural Research - IPA. In 2003, Embrapa released cultivars BRS-228 Sapota and BRS-227-Sapoti IPA-Curu (Miranda *et al.*, 2008).

The Sapodilla culture demands reasonable amount of mineral fertilizers to achieve a satisfactory productivity, due to high amounts of nutrients that plants derive from the soil and the low fertility of Northeastern soil where it is grown. Nitrogen is essential constituent of plants, as part of the composition of aminoacids, enzymes, nucleic acids, and chlorophyll, increasing the photosynthetic capacity of the plants.

Research results have shown that nitrogen fertilization and fruit development stage influence the production, nutritional composition and quality of the fruit. Work carried out with other cultures have shown positive results of the nitrogen influence on the physical and physicochemical characteristics of fruits (Medeiros *et al.*, 2004). While Miranda *et al.* (2008), found that during the development of sapodilla fruits, there is an increase of soluble sugars, the fresh weight and size, respectively.

However, there are no reports in the literature on the influence of nitrogen fertilization on the sapodilla physical and physicochemical characteristics and antioxidant potential. The aim of this research was to evaluate the effect of nitrogen fertilization on the physical and physicochemical characteristics and antioxidant potential of sapodilla at different stages of development.

Material and methods

An experiment was conducted in a commercial planting of the Norfruit farm, located in the rural community of Pau Branco, Mossoro/RN, Brazil. This municipality is located at 5º 11» south latitude and 37º20» east longitude and at 18 m.a.s.l. The climate is semi-arid and according to Koppen classification is categorized as "BSwh", dry and very hot, with two seasons: dry, which usually from June to January, and a rainy season from February to May. During the experiment, the weather station Mossoro presented an average annual temperature data of 27.6°C, average relative humidity of 68% and 765.8 mm of rainfall.

The orchard used in this study consisted of grafted plants from sapodilla cv. Itaparema 31, with eight years of age, planted with a row spacing of 7 x 7 m, with 225 plants per hectare, which performed a good good plant health aspect and an adequate sprinkler irrigation system.

A randomized block design was adopted with two factorial schemes as follows: the first 5 x 6 with lost parcels, consisting of five doses of nitrogen (N) (0, 300, 600, 900 and 1200 g of N. plant^{-1}, respectively.) and six stages of fruit development (90, 120, 150, 180, 200, 208 days, respectively.) The second, 2 x 3 composed of two nitrogen doses (0; 600 g of N.plant^{-1}) and three developmental stages (120, 180 and 208 days, respectively.), each with five replicates. In field condition, each plot consisted of five plants used in the useful portion of three central plants. The parcels were isolated from each other throughout a parallel plating line on both sides.

Before the treatments application, sapodilla plants received a fructification pruning. In fact, Nitrogen rates were applied in the form of urea (45% N), divided in intervals of 30, 60 and 90 days, respectively. The plants received the same dose of potassium and phosphorus for all treatments using 890 g of potassium chloride, and 682 g of granulated mono ammonium phosphate, twice per plant parceling. Subsequently, the choice of fertilizer dosages was performed according to a previous research conducted by Lederman *et al.* (2001), with recommended fertilizer for sapodilla. After fertilization and identification of sapodilla inflorescences, the fruits were marked in the field with 10 to 15 mm of cross-sectional diameter, which have allowed an uniformity in fruit development. The fruit harvest was performed at 90, 120, 150, 180 and 200 days, respectively after field marking. After 200 days twice the number of fruit was collected, half of which were analyzed on the collection day and the other half stored at room temperature (25 ± 2°C and 58 ± 5% RH) and analyzed after eight days of storage with respect to a full ripening (208 days).

Therefore, for treatments with doses of 0, 300, 600, 900 and 1200 g of N plant^{-1} in all stages of development, the following evaluations were carried out: transverse and longitudinal diameters using a digital caliper and the results expressed in cm; fresh weight of the intact fruit, with the aid of a semi-analytical scale and expressed in grams; Soluble solids content of processed pulp obtained by a digital refractometer, and the result is expressed in%; and vitamin C content, determined by the Tilman method, by titration, and the results expressed in mg 100g^{-1} of ascorbic acid.

The control treatments (dose 0) and at doses of 600 g of N. plant^{-1} with the fruits harvested

at 120 and 180 days after field marking and 208 days (with full ripening), have allowed to establish analysis as follows: total soluble sugars, which were measured by the Anthrone method, according to the methodology described by Yemm & Willis (1954). Reducing sugars, by extracting with distilled water and the subsequently determination was performed according to Miller (1959). Anthocyanin content and yellow flavonoids, measured according to Francis (1982); total extractable polyphenols, determined according to the methodology described by Larrauri *et al.* (1997); and total antioxidant activity, as determined by the DPPH method described by Brand-Williams *et al.* (1995), based on the DPPH (2,2-diphenyl-1-picryl-hydrazyl) sequestration by antioxidants, which producing a decrease in absorbance at 515 nm.

Analysis of variance was performed to test the treatments and analysis curve fitting to the figures. For data analysis, SAS (Statistical Analysis System) software® and Bioestat 5.0® were used. To identify significant difference among treatments and statistical significance for all comparisons was made at $p<0.05$. Tukey's multiple range test was used to compare the mean values of treatments. The relationship between antioxidant activity and bioactive compounds was obtained by Pearson correlation.

Results

Fresh fruit mass increased to 200 days, where all N dosages were tested, control treatment exhibited the lowest mass (174.93 g) and the highest dose of 600 g of N.plant^{-1} (204.75 g). After eight days of storage conditions (temperature 25 ± 2°C and RH 58 ± 5%), the fruit mass was decreased (Figure 1).

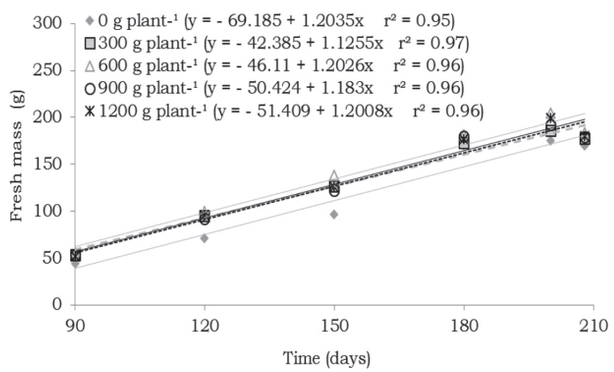

Figure 1. Fresh mass of sapodilla fruit subjected to fertilization with five doses of nitrogen and harvested at different stages of development.

The fruits reached their maximum size at 200 days, with an average longitudinal diameter of 6.89 cm and 6.98 cm across. Throughout development, transversal and longitudinal diameter ratio was close to 1.0, which indicates a fruit with rounded shape (Figure 2).

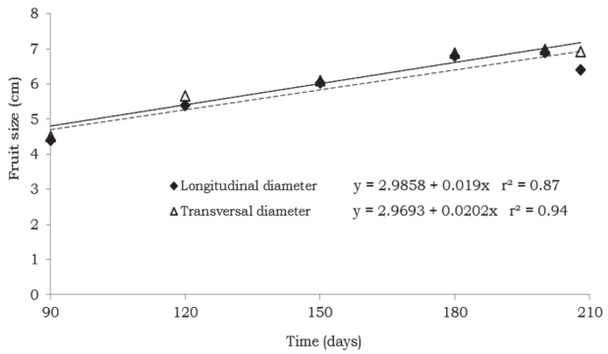

Figure 2. Longitudinal and transversal diameters of sapodilla fruit subjected to fertilization with five doses of nitrogen and harvested at different stages of development.

During the sapodilla development, the soluble solids reached a maximum of 19.5% at 208 days of development when fruits had achieved an entire ripening, i.e., suitable for consumption (Figure 3). Among the tested doses, the control treatment showed the highest soluble solids (18.30%) with a slight decrease to 17.5% with the increase of N rates (Figure 4).

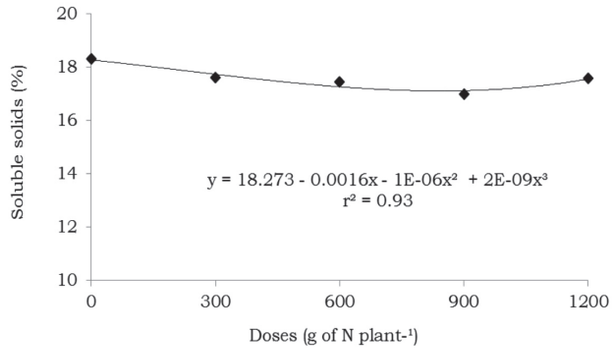

Figure 3. Soluble solids of sapodilla fruit subjected to fertilization with five doses of nitrogen and harvested at different stages of development.

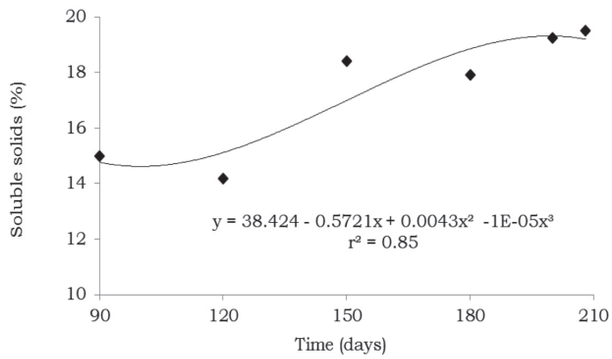

Figure 4. Soluble solids of sapodilla fruit subjected to fertilization with five doses of nitrogen and harvested at different stages of development.

Early in development, the sapodilla were extremely firm, making it impossible to determine the firmness with a penetrometer. The fruit firmness decreased with the advancing of the stages of development, obtaining values of 107.8N, 97.5N and 33.54N to 180, 200 and 208 days of fruit development, respectively. In addition, it was observed a loss of firmness with an increasing nitrogen levels (Figure 5).

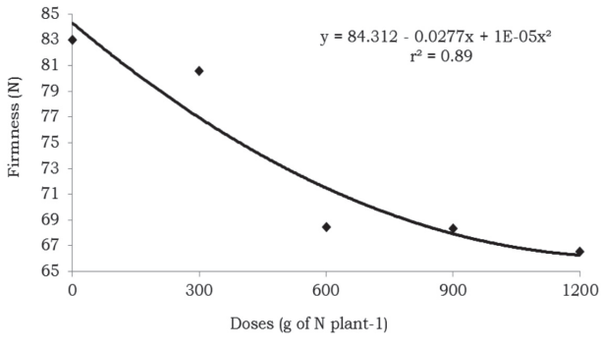

Figure 5. Firmness of sapodilla fruit subjected to fertilization with five doses of nitrogen and harvested at different stages of development.

The vitamin C content decreased with fruit development timing, on the other hand, there was no effect of N rates for this variable. At 90 days, the sapodilla contained a higher content of vitamin C (15.90 mg $100g^{-1}$), reaching 208 days of development with values around 10.38 mg $100g^{-1}$ (Figure 6).

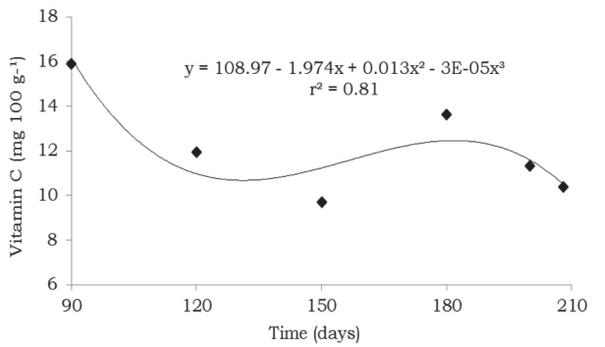

Figure 6. Vitamin C of sapodilla fruit subjected to fertilization with five doses of nitrogen and harvested at different stages of development.

The levels of total and reducing sugars were higher in fruits evaluated at 180 and 208 days, which did not differ amongst themselves. The fruits submitted to fertilization with 600 g of $N.plant^{-1}$ had achieved higher sugar content than sapodilla fruit without any fertilization treatment (Table 1). The anthocyanins (Table 1) and flavonoids (Table 2) content, decreased with an increasing of fruit development and nitrogen fertilization.

Table 1. Values of soluble sugars (%), reducing sugars (%), anthocyanin (mg $100g^{-1}$), total antioxidant activity (g of fruit/g DPPH) of sapodilla fruit subjected to fertilization with five doses of nitrogen and harvested at different stages of development.

Variable	Doses	Time		
	(g of N. $plant^{-1}$)	120 days	180 days	208 days
Soluble sugars	0	6.69 Bb	11.76 Ab	12.72 Ab
	600	6.89 Ba	13.75 Aa	15.20 Aa
Reducing sugars	0	5.23 Bb	8.42 Ab	11.04 Ab
	600	6.39 Ba	10.9 Aa	12.87 Aa
Total anthocyanin	0	8.93 Aa	3.93 Ba	3.43 Ca
	600	4.95 Ab	3.23 Bb	2.19 Cb
Antioxidant activity	0	559.63Aa	919.82 Ba	1327.55 Ca
	600	561.00Aa	985.61 Ba	1300.96 Ca

Uppercase letters represent statistical difference between the maturity stages and lowercase letters represent statistical difference between tested doses at $p \leq 0.05$ by Tukey test.

Table 2. Values of yellow flanonoids (mg $100g^{-1}$) e total extractable polyphenol (mg $100g^{-1}$) of sapodilla fruit subjected to fertilization with five doses of nitrogen and harvested at different stages of development.

Time (days)	Yellow flavonoids	Total extractable polyphenol
120	34.22 A	976.35 A
180	23.16 B	792.42 B
208	11.52 C	110.11 C
Doses (g de N $plant^{-1}$)	Yellow flavonoids	Total extractable polyphenol
0	25.01 a	612.02 a
600	20.93 b	640.57 a

Uppercase letters represent statistical difference between the maturity stages and lowercase letters represent statistical difference between the doses tested at $p \leq 0.05$ by Tukey test.

The total antioxidant activity (Table 1) and total extractable polyphenol content (TEP) (Table 2), decreased with development, which were not affected by nitrogen fertilization.

There was a positive correlation between the antioxidant activity and bioactive compounds evaluated. Among the bioactive compounds analyzed, vitamin C showed the lowest correlation with the antioxidant activity, whereas the content of total extractable polyphenols showed the highest correlation with the antioxidant activity of fruits (Table 3).

Table 3. Pearson correlation between bioactive compounds and antioxidant activity by DPPH method of sapodilla fruits subjected to fertilization with five doses of nitrogen and harvested at different stages of development.

	FL	ANT	PET	Vit. C	DPPH
ANT	0.8437*				
PET	0.8873*	0.5960			
Vit. C	0.8085	0.7304	0.7214		
DPPH	-0.9368**	-0.7403	-0.9404**	-0.6475	

Discussion

The mass is an extremely important attribute in the marketing of fruits, which can be used as a variable for determining the price of the product due to consumer does not pay for the unit, but per fruit kilogram.

The sapodilla fruits produced in the present study, had achieved a greater mass at two hundred days of development, occurring a reduction after the harvest and storage. These results are comparable in variability to the report by Miranda *et al.* (2008), who found sapodilla fruits with 180 days, had achieved an average weight of 127g, decreased to 113g after climacteric condition. This reduction is one of the early symptoms of water loss, caused by the loss of moisture and reserve material through plant uptake and perspiration (Silva *et al.*, 2009).

The fruit format and mass are very important visual attributes for consumers at the time of purchase. The obtained fruits in this study presented a round format, similar to those found in previous work. Costa *et al.* (2000), also found a tendency of the sapodilla cv. "Itapirema-31" to have a round format with a transverse diameter (6.5 cm) higher than longitudinal (6.2cm).

As can be seen in Figure 3, soluble solids were not very influenced by nitrogen fertilization. In studies with other fruit such as passion fruit (*Passiflora edulis* Sims.) (Venâncio *et al.*, 2013), papaya (*Carica papaya* L.) (Souza *et al.*, 2009), guava (*Psidium guajava* L.) (Amorim *et al.*, 2015), a low influence of nitrogen fertilization on the levels of fruit soluble solids, was detected in accordance with the obtained results in this research, which were pooled for subsequent analyses.

The fruit firmness decreased with an increasing of nitrogen fertilization. This was probably due to a nitrogen excess which have allowed a decreasing in the mesocarp tissue ability to accumulate calcium, causing less fruit firmness, since calcium favors the linkage between pectin chains, which give fruit firmness (Miqueloto *et al.*, 2011).

Analyzing sapodilla, Isabelle *et al.* (2010), found vitamin C content around 10.14 mg 100g-1, similar to the reported in this study.

The sugars content increased with nitrogen supplying (Table 1). Lemos *et al.* (2008), report the existence of an interaction between nitrogen metabolism and photosynthesis, giving photosynthetic organisms a better production of photoassimilates in the nitrogen presence, which have allowed an increasing in the sugar content.

Nevertheless, an observed decreasing in the polyphenols content with fruit ripening can be attributed to the fact that during the fruit development, two of the most important polyphenols, chlorogenic acid and flavonoids have suffered a detriment (Awad *et al.*, 2001). In addition, due to an increasing in the phenolic soluble condensation, making them insoluble for a strong linkage into other cellular components, which is therefore not detected. Fernández *et al.* (2011), observed that the anthocyanin content of the sapodilla decreased over ripening, this fact may occur due to the use of anthocyanins by the polyphenol oxidase to form quinones.

Concordantly with the obtained results in the present study, Shui *et al.* (2004), observed that the sapodilla antioxidant ability, was decreased during storage period. The authors add that the best time to consume the sapodilla is between the third and fourth day of storage due to sapodilla fruits had achieved a good organoleptic quality and still performs a high antioxidant activity and total phenolic contents. Therefore, Reyes *et al.* (2005), found that the total phenolic content decreased during the sapodilla development process.

According to Shui *et al.* (2004), the antioxidant activity of sapodilla strongly correlates with total phenolic contents, agreeing with the data from this research.

Conclusions

Nitrogen fertilization and fruit ripening stage influence the quality and antioxidant potential of sapodilla fruit.

The dose of 600 g of N.plant^{-1} provided a production of sapodilla fruit with higher sugar content and fresh weight, reaching greater mass and sugar content, at 200 and 180 days of development, respectively.

Given these concerns, bioactive compounds (total polyphenols, vitamin C, flavonoids and anthocyanins) and sapodilla antioxidant activity, had achieved a decreasing with the further fruit development, owing to its high natural process of fruit senescence. The antioxidant activity of the sapodilla fruit is more related to the high content of total polyphenols.

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