

Physicochemical characteristics, minerals, phenolic compounds, and antioxidant capacity in fig tree fruits with macronutrient deficiencies

Carlos A. GARZA-ALONSO¹, Guillermo NIÑO-MEDINA¹,
Adriana GUTIÉRREZ-DÍEZ¹, Josué I. GARCÍA-LÓPEZ²,
Rigoberto E. VÁZQUEZ-ALVARADO¹, Alfredo LÓPEZ-JIMÉNEZ³,
Emilio OLIVARES-SÁENZ^{1*}

¹Universidad Autónoma de Nuevo León, Facultad de Agronomía, Francisco Villa s/n col. ExHacienda el Canadá, Gral. Escobedo, N.L., México; carlos.garza.alonso@gmail.com; emolivares@gmail.com (*corresponding author); guillermo.ninomd@uanl.edu.mx; mcgudiez@aol.com; rvazquez.alvarado@gmail.com

²Universidad Autónoma Agraria Antonio Narro, Departamento de Fitomejoramiento, Centro de capacitación y desarrollo en tecnología de semillas, Calzada Antonio Narro 1923 col. Buenavista, Saltillo, Coah., México; g.lopezj90@gmail.com

³Colegio de Postgraduados, Recursos Genéticos y Productividad-Fruticultura, Carretera México-Texcoco Km 36.5, Montecillo, Texcoco, Edo. de Méx., México; lopezja@colpos.mx

Abstract

In this research, effects of macronutrient deficiency (N, P, K, Ca, and Mg) on the production, physicochemical characteristics, minerals, phenolic compounds, and antioxidant capacity of fig fruits (*Ficus carica* L.) were evaluated using the missing element technique in a controlled hydroponic system under greenhouse conditions. N-deficient plants had no fruit production, while fruits with absence of P, K, and Ca were the most affected in terms of size, weight, and physicochemical characteristics. On the other hand, the concentration of minerals was significantly different ($p < 0.05$), finding some interactions of synergism and antagonism between ions. Phenolic compounds increased in fruits with P and Ca deficiency, as well as the antioxidant capacity DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) in the fruits of the treatment -Ca. Regarding the FRAP (ferric reducing antioxidant power) test, higher values were found for all treatments without minerals (-P, -K, -Ca, and -Mg) with respect to the control. The results obtained explain the responses of the fig tree subjected to nutritional deficiencies.

Keywords: condensed tannins; *Ficus carica* L.; flavonoids; fruit quality; plant nutrition

Introduction

The fig tree (*Ficus carica* L.) is a deciduous plant native to the Mediterranean region, whose cultivation has been increasing in recent years (FAO, 2019). The fruits are appreciated for their high commercial value (USDA, 2019), high sugar content (Crisosto *et al.*, 2010), as well as high phenolic compounds and antioxidant power (Vallejo *et al.*, 2011; Harzallah *et al.*, 2016). Furthermore, various studies have found that fig leaves have

hypoglycemic properties (Deepa *et al.*, 2018); the fruits, roots, stems, and leaves of the plant have other beneficial effects such as anticancer and antiseptic properties (Badgular *et al.*, 2014).

Plant nutrition is one of the most important factors for crop production. Nutrients play specific functions in plants, when these elements are at low levels, certain specific physiological processes are affected, such as photosynthesis and cellular respiration (Karthika *et al.*, 2018); therefore, nutritional deficiencies affected vegetative growth, production, and fruit quality, specifically the absence of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) (Pandey, 2015). The missing element technique allows studying the effects of nutrient deficiency; this experimental method consists in removing from the nutrient solution the element to be analyzed (Kathpalia and Bhatla, 2018). This technique has been used in different species, such as the work reported by Mota and Rocha (2014), who found that the size and weight of pineapple fruits (*Anana comosus*) decreased when the plants suffered some deficiencies of N, P, and K in a 57.59, 26.78, and 22.8%, respectively. The same study found a 200% increase in Vitamin C content in N-deficient fruits, compared to full fertilization. On the other hand, Nava *et al.* (2008) studied the effects of N and K deficiencies in apples, finding negative effects on some physicochemical parameters, such as titratable acidity and °Brix, which increased linearly with increasing doses of these elements. Furthermore, various authors have reported the effects of macronutrient suppression on the concentration of phenolic compounds and antioxidant capacity in plants such as *Lens culinaris* (Chandra and Karmoker, 2011), *Daucus carota* (Singh *et al.*, 2011), and *Morus alba* (Kumar *et al.*, 2007), finding differences between species.

For the fig tree, Garza-Alonso *et al.* (2019) reported the effects of deficiency of N, P, K, Ca, and Mg on the vegetative growth and minerals concentration in leaf, root, and stem; however, few studies have been conducted on the effects of macronutrient deficiencies in the quality of fig fruits. Esref *et al.* (2008) studied the effects of different doses of Ca, where the average fruit weight decreased from 16.24 to 14.43 g when this element was not applied, which also produced a 34% reduction in yield per plant. In other studies, Soliman *et al.* (2018) carried out similar evaluations with K, finding a reduction of 28.2% in fruit weight and 10.34% in the total soluble solids content when K was absent in the nutrient solution. On the other hand, Holstein *et al.* (2017) reported an 18.7% reduction in fruit yield between plants with and without applications of K. Gaaliche *et al.* (2019) evaluated the effects of the foliar application of K against the non-application of the same element on the concentration of phenolic compounds, finding a decrease of 14.33% in the content of total phenols, 48% reduction for total flavonoids, as well as a 34.44% increase in the antioxidant capacity DPPH by not applying K. For N, P, and Mg, the available information is very limited, and in most cases, not enough variables are reported to allow decisions to be made and results to be concluded.

Based on the above, the objective of the present investigation was to evaluate the effects of deficiency of N, P, K, Ca, and Mg on production, physicochemical characteristics, mineral content, phenolic compounds concentration, and antioxidant capacity of fig fruits.

Materials and Methods

Experiment location and plant material

The experiment was carried out under controlled conditions in a greenhouse, with an area of 1,000 m² and 4.5 m in height at the lateral sides, its highest part being 7 m. The average daily temperature was 25 °C and a relative humidity of 75%. The plants correspond to the 'Adriatic' cultivar, the fruit is white (ripening with green epicarp) and dark red flesh. The size is medium, approximate average weight of 30 g.

Induction of nutritional deficiencies

The missing element technique was used to induce nutritional deficiencies in plants, which consisted of removing the element under study from the nutrient solution for each case (-N, -P, -K, -Ca, -Mg). The substrate used was perlite, an inert material with a neutral pH and without mineral nutrient content (Papadopoulos *et*

al., 2008). The experimental unit consisted of a 19 L pot with a single-stemmed plant. The root system was washed with distilled water to remove the remains of soil before placing the plants in the pots. Irrigation was supplied automatically through use of timers, applying eight daily pulses of 60 mL per pot. The treatments were: T1 - Complete nutritional solution (Control); T2 - Solution without nitrogen (-N); T3 - Solution without phosphorous (-P); T4 - Solution without potassium (-K); T5 - Solution without calcium (-Ca); and T6 - Solution without magnesium (-Mg).

Preparation of nutritional solutions

The nutrient solution proposed by Hewitt was selected for the preparation of the nutritional solutions, which was evaluated in the fig tree by Sevil *et al.* (2007). The nutrient solution consisted of the following element concentrations (mg L^{-1}): N 168, P 41, K 156, Ca 160, Mg 36, S 48, Fe 2.8, Mn 0.55, B 0.54, Cu 0.064, Zn 0.065, and Mo 0.048. The preparation of the solutions was carried out using rainwater, with an electrical conductivity of 0.05 dS m^{-1} . The water analysis did not identify minerals that affected the investigation. For the preparation of the nutritive solutions, fertilizer sources lacking each element under study were used.

Agronomic parameters and physicochemical characteristics of the fruit

The evaluation of the agronomic parameters included: Number of fruits per plant, fresh weight, yield per plant, fruit polar and equatorial diameter.

The flesh and peel color evaluations were determined directly with a portable colorimeter (Konica Minolta BC-10, Konica Minolta, Japan). Color parameters were obtained using the CIELAB (L^* , a^* , b^*) and CIELCH (L^* , C^* , h^*) color systems according to the Commission Internationale De L'eclairage (CIE, 2004). L^* defines luminosity (0 black, 100 white), a^* indicates red (a^* positive) or green (a^* negative), b^* indicates yellow (b^* positive) or blue (b^* negative), C^* (chroma) saturation level, and h (hue angle: 0° =red, 90° =yellow, 180° = green, 270° =blue). Color visualization was obtained using the online software ColorHexa (2019) using the L^* , a^* , and b^* values.

Total soluble solids content ($^\circ\text{Brix}$) was performed by taking a portion of the pulp, which was placed in a digital refractometer (Atago Pen-pro, Atago Inc., USA), with a range of 0-85%. Titratable acidity was determined by weighing 5 g of pulp, which were processed with 40 mL of distilled water in a mixer (Oster blender M4655-813 / 465-42 Sunbeam, Mexico) for 30 s. The extract was filtered through organza cloth. The pH was measured using a potentiometer (pH / ORP Meter HI 2221, Mexico). Titratable acidity (TA) was determined by method 942.15 of the Official Association of Analytical Chemistry (AOAC, 2000), with 0.1 M NaOH at pH 8.2 using phenolphthalein as an indicator. Titratable acidity was reported as a percentage of citric acid in fresh weight (g/100g).

Determination of minerals

Fruits samples were dried in a Yamato DX 602C oven (Yamato Scientific Co, Japan) at 60°C for 72 h. The resulting material was crushed and subjected to acid digestion in a mixture of perchloric acid and nitric acid (Alcántar and Sandoval, 1999).

N was quantified by the micro Kjendahl method according to the Bremner (1965) methodology. Fruits concentrations of P, K, Ca, Mg, Fe, B, Mn, Zn, and Cu was carried out using the extract of acid digestion by means of a coupled plasma induction atomic emission spectrometer (ICP-AES Agilent 725- ES, Agilent Technologies, USA).

Extraction of soluble (free) phenolic compounds

Fruits were separated into pulp and peel and 5 g of sample for each part were weighed and processed in a mixer (Oster Blender M4655-813 / 465-42 Sunbeam, Mexico) with 50 mL of 80% methanol for 30 s. Subsequently, the mixture was filtered with organza fabric to separate the remaining insoluble matter, which was used for the extraction of bound phenolic compounds. The resulting extract was placed in 50 mL Falcon

tubes and spin at 4500 rpm for 5 min. The supernatant was recovered and stored in the dark at -20 °C to carry out measurements of phenolic compounds and antioxidant capacity.

Extraction of bound phenolic compounds

Bound phenolic compounds were removed by suspending the remaining insoluble matter in test tubes, to which 5 mL of 2M NaOH was added and purged with argon for 30 s. The mixture was then stirred for 2 h. Subsequently, the pH was adjusted to 2.5 with HCl and centrifuged at 4500 rpm for 5 min. The supernatant was recovered, and the bound phenolic compounds were extracted with 5 mL of ethyl acetate (2 times). The ethyl acetate extracts were combined and placed on a rotavapor (Yamato RE201, Yamato, Japan) for 10 min to evaporate the ethyl acetate. Once evaporated, the sample was resuspended with 3 mL of 80% methanol. The extracts obtained from soluble and bound phenolic compounds were used for the tests of total phenols, total flavonoids, condensed tannins, and antioxidant capacity (DPPH, ABTS and FRAP) (López-Contreras *et al.*, 2015).

Determination of total phenols

For the determination of total phenolic compounds, 0.2 mL of each extract was taken and mixed with 2.6 mL of distilled water, and 0.2 mL of Folin-Ciocalteu reagent. After 5 min, 2 mL of 7% Na₂CO₃ was added, and the solution was stirred for 30 s. Subsequently, the mixture was allowed to stand for 90 min in the dark, and the absorbance was measured at a wavelength of 750 nm. Calculations were performed using a gallic acid calibration curve in a range of 0 to 200 mg kg⁻¹. The concentration of total phenols was reported as equivalent milligrams of gallic acid per kilogram of sample (mg GAE kg⁻¹).

Determination of total flavonoids

Total flavonoids concentration was determined by means of the AlCl₃-NaNO₂-NaOH reaction, for this 0.2 mL of the extract was taken and 3.5 mL of distilled water were added. Subsequently, 0.15 mL of 5% NaNO₂, 0.15 mL of 10% AlCl₃, and 1 mL of 1M NaOH were added; the above at 5 min intervals between each addition of reagents. The reaction was left for 15 min, and then the absorbance was measured at 510 nm wavelength. Results were reported as milligrams of catechin equivalent per kg of sample (mg CatE kg⁻¹), calculated from a catechin calibration curve in a range of 0 to 200 mg kg⁻¹.

Determination of condensed tannins

Tannin content was determined using a Vanillin-H₂SO₄ reaction, for which 0.25 mL of the extract, 0.65 mL of 1% Vanillin, and 0.65 mL of 25% H₂SO₄ were taken (both were dissolved in methanol). The mixture was allowed to stand for 15 min at 30 °C, and the absorbance was subsequently measured at a wavelength of 500 nm. Calculations were performed using a catechin calibration curve in a range of 0 to 200 mg kg⁻¹. Results were reported as milligrams of catechin equivalent per kilogram of sample (mg CatE kg⁻¹).

Antioxidant capacity DPPH, ABTS and FRAP

DPPH (2,2-diphenyl 1-picrylhydrazyl) antioxidant capacity was evaluated using a 60 µM working solution in 80% methanol, which was adjusted to an absorbance of 0.99 at 517 nm wavelength. The test was performed by mixing 50 µL of the extract with 1.5 mL of DPPH working solution, which was left to stand for 30 min in the dark, and the absorbance was determined.

The antioxidant capacity ABTS (2,2-azino-bis (3-ethylbenzothiazolin) -6-sulfonic acid)) was determined using a working solution obtained by mixing 1 mL of ABTS 7.4 mM and 1 mL of K₂S₂O₈, which was left to react for 12 hours in the dark. The test was performed by adding 50 µL of the extract and 1.5 mL of the ABTS working solution. The mixture was allowed to stand for 30 min, and the absorbance was determined with a wavelength of 734 nm.

The antioxidant capacity FRAP (ferric reducing antioxidant power) was determined using a working solution prepared with 300 mM $C_2H_3NaO_2 \cdot 3H_2O$, 10 mM TPTZ (2,4,6-tripyridyl-1,3,5-triazine, in 40 mM HCl), and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio (V:V:V). The assay was performed by adding 50 μ L of the extract and 1.5 mL of the FRAP working solution. The reaction was allowed to stand for 30 min in the dark at 37 ° C. Subsequently, the absorbance was measured at 593 nm wavelength. The antioxidant capacity for the DPPH, ABTS, and FRAP assays were reported in Trolox equivalent micromoles per kilogram of sample (μ molTE kg^{-1}), based on the Trolox calibration curve (6-hydroxy-2,5,7 acid, 8-tetramethylchroman-2-carboxylic) in a range of 0 to 500 μ mol kg^{-1} .

Experimental design and statistical analysis

The experiment was established using a completely randomized experimental design with six treatments and three repetitions per treatment; the experimental unit was one plant per pot. The results were reported as mean \pm standard deviation, the statistically significant differences between the treatments were identified with an ANOVA, and the means of the treatments were compared by the Tukey method ($p < 0.05$), using the statistical package SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Agronomic parameters

Plants with the -N treatment did not produce fruits, therefore, only the effects of the -P, -K, -Ca, and -Mg treatments were studied. The analysis of variance showed significant differences ($p < 0.05$) for all the evaluated agronomic variables (Table 1). Fruit weight was principally affected by Ca deficiency, reducing by 40.3% (18.52 ± 1.49 g) compared to control (31.01 ± 0.86 g). The -P treatment followed -Ca for reduction in fruit weight with a reduction of 33.7% (20.56 ± 0.53 g), compared to the control. Similar results were found for the number of fruits, since the -Ca treatment affected 60.7% (5.50 ± 0.70) with respect to the control (14.00 ± 0.01); the same pattern was presented for the -P treatment with a decrease of 35.7%, while the other treatments were not different statistically from the control. The same trend was observed for fruit yield per plant; The -Ca, -P, and -K treatments presented reductions of 75.1, 73.8, and 36.1%, respectively.

Table 1. Effect of macronutrient deficiencies on agronomics characteristics of fig tree fruits

Treatment	Fruit weight (g)	Fruit number	Yield (g plant ⁻¹)	Polar diameter (mm)	Equatorial diameter (mm)
Control	31.01 ± 0.86 a	14.00 ± 0.01 a	449.07 ± 30.81 a	41.06 ± 1.25 a	39.27 ± 1.63 a
-P	20.56 ± 0.53 bc	9.00 ± 2.82 ab	117.57 ± 45.29 c	36.81 ± 1.24 b	34.02 ± 2.47 ab
-K	24.75 ± 1.65 b	11.50 ± 2.12 a	286.79 ± 21.17 b	38.05 ± 0.03 ab	35.03 ± 0.62 ab
-Ca	18.52 ± 1.49 c	5.50 ± 0.70 b	111.72 ± 21.78 c	35.94 ± 1.54 b	32.25 ± 2.93 b
-Mg	31.63 ± 0.39 a	13.50 ± 0.70 a	409.15 ± 41.01 ab	41.84 ± 1.19 a	35.75 ± 1.86 ab

Values are the mean of treatments ($n=3$) \pm standard deviation. Different letters in each column means that the treatments were statistically different (Tukey, $p < 0.05$).

On the other hand, -Ca and -P treatments decreased the polar diameter of the fruits, the reductions were 12.8% (35.94 ± 1.54 mm) and 10.3% (36.81 ± 1.24 mm), respectively, with respect to the control (41.06 ± 1.25 mm). Finally, the equatorial diameter of the fruit with the -Ca treatment reported the greatest reduction of 17.87% (32.25 ± 2.93 mm) with respect to the control (39.27 ± 1.63 mm), without finding statistical differences in the other treatments (Table 1).

The nutrient absorption curves for fig cultivation developed by Mendoza *et al.* (2019) showed that the plant increases the demand for Ca, P, and K at the beginning of fruit formation, which explains the smaller size and weight of the fruits obtained in this research.

P is an important constituent of nucleic acids and ATP, so it is involved in all energy transfer reactions in cells. Furthermore, this element is involved in photosynthesis, sugar translocation, and nutrient movement in plants (Hawkesford *et al.*, 2012; Alcántar *et al.*, 2016; Karthika *et al.*, 2018). The lower weight, quantity, and size of fruits in plants without P are explained by the importance of this element in plant physiology.

Large amounts of K are found as free ion at the level of vacuoles and cytoplasm, thereby generating an osmotic pressure and thus causing cell turgor. On the other hand, K participates in the reactions of photosynthesis and cellular respiration, in addition to promoting an efficient translocation of photosynthates from the leaves to other organs of the plant, mainly fruits (Alcántar *et al.*, 2016). The accumulation of photoassimilates (sucrose, glucose, and fructose) has been shown in rice plants with P, K, and Ca deficiencies (Kobayashi *et al.*, 2013). All of the above explains the results found in the present investigation.

Soliman *et al.* (2018) evaluated K doses on the quality of fig fruits, finding a 28.2% reduction in fruit weight in the treatment without the addition of potassium fertilizers. The same trend was found in the work of Holstein *et al.* (2017), who reported greater size and fruit yield when applying K compared to the null application of this element, the decrease in yield was 18.7% in plants with absence of K.

Ca is an important element for the formation of the cell wall and membrane, in addition to participating in the processes of cell division and multiplication (Hawkesford *et al.*, 2012; Alcántar *et al.*, 2016). The first two phases of fruit growth consist of cell proliferation and thickening (Agustí, 2010), so a Ca deficiency affects these processes. The above explains the results found in this study regarding the lower weight and size of fruits of the -Ca treatment. In another study, Esref *et al.* (2008) found that Ca deficiency decreased the average fruit weight from 16.24 to 14.43 g when not applying this element, as well as a 34% decrease in fruit yield per plant, which represents the same effect found in this investigation.

Physicochemical characteristics of the fruit

The results associated with the physicochemical quality of the fruit showed significant differences ($p < 0.05$) in all the variables evaluated (Figure 1). For the total soluble solids content (°Brix), the lowest value was observed in the -K treatment (15.21 ± 2.62), which represented a decrease of 17.73% with respect to the control (18.49 ± 0.87), while the fruits of the -Ca treatment presented an increase of 21.36% (22.44 ± 1.05), without finding significant statistical difference between the other treatments (Figure 1a). On the other hand, the titratable acidity decreased in the -K and -Mg treatments by 45% (0.22 ± 0.03) and 37.5% (0.25 ± 0.02), respectively, with respect to the control (0.4 ± 0.01), on the other hand, The -P and -Ca treatments were statistically the same (Figure 1b). The same trend was found for the pH of the fruit, where the -K treatment resulted in the lowest value, presenting a reduction of 10.20% (4.84 ± 0.12) with respect to the control (5.39 ± 0.07), without finding differences with the other treatments (Figure 1c).

The decrease in the total soluble solids content (°Brix) in fruits with macronutrient deficiency (P and K) is explained due to the accumulation of photosynthates in the leaves, as well as the low mobilization towards the fruits, as explained above. The reduction of °Brix in fruits with K deficiency was also reported by Soliman *et al.* (2018), finding a decrease of 10.34% with respect to the control. On the other hand, the increase in the amount of total soluble solids in the fruits of the -Ca treatment is explained by the fact that the plants resulted with the smallest size and quantity of fruits (18.52 ± 1.49 and 5.50 ± 0.70), causing an effect of sugar concentration in them due to the greater amount of photosynthates accumulated in the fruit per unit of weight.

The effects found in terms of physicochemical characteristics have been reported in other fruits, such as orange (*Citrus* spp.) (Quaggio *et al.*, 2011) and apple (*Malus domestica*) (Nava *et al.*, 2008).

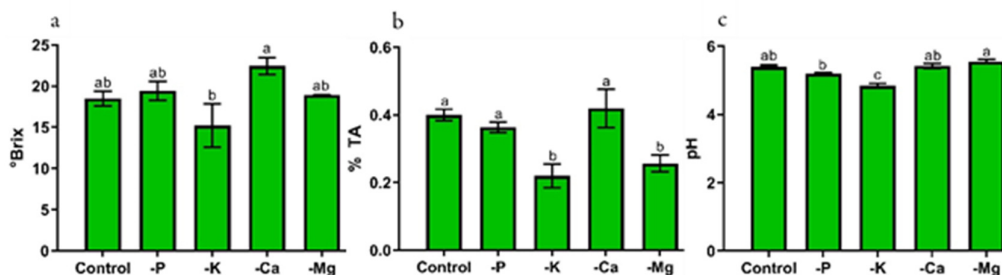


Figure 1. Total soluble solids content (°Brix) (a), titratable acidity (b), and pH (c) of fig tree fruits with macronutrient deficiencies. Values are the mean of treatments ($n=3$). Bars represent the standard deviation of mean.

Different letters in each column means that the treatments were statistically different (Tukey, $p < 0.05$).

Chromatic characteristics of the fruits

Results of chromatic characteristics of the fruits showed significant differences ($p < 0.05$) in all the variables (Table 2). In the case of pulp color, the highest value of L^* was observed in the -K treatment (35.66 ± 0.98), however, no significant differences were found between the other treatments. The aforementioned indicates greater luminosity in the fruit pulp as they are subject to K deficiency. On the other hand, results indicated that the lowest values in b^* were found in the -P, -Mg, and -Ca treatments (3.26 ± 0.51 , 4.70 ± 0.14 and 5.20 ± 0.14) respectively, compared to the control (7.55 ± 0.49). The same trend was found in C^* , since the lowest values were obtained in the -Ca and -P treatments (9.35 ± 0.77 and 9.66 ± 0.51), with respect to the control (12.10 ± 0.14). On the contrary, in h^* , it is only observed that the -P treatment obtained the lowest value (19.90 ± 3.11), compared to the other treatments that did not show a significant difference.

Table 2. Chromatic characteristics in pulp and peel of fig tree fruits with macronutrient deficiencies

Treatment	L^*	a^*	b^*	C^*	h	View
Pulp						
Control	30.86 ± 1.28 ab	8.83 ± 1.05 ns	7.55 ± 0.49 a	12.10 ± 0.14 a	36.93 ± 3.81 a	
-P	30.06 ± 1.96 b	9.10 ± 0.51 ns	3.26 ± 0.51 d	9.66 ± 0.51 b	19.90 ± 3.11 b	
-K	35.66 ± 0.98 a	9.99 ± 0.79 ns	6.30 ± 0.00 ab	11.23 ± 0.50 ab	36.93 ± 4.78 a	
-Ca	33.05 ± 1.76 ab	7.75 ± 1.06 ns	5.20 ± 0.14 bc	9.35 ± 0.77 b	32.23 ± 4.42 a	
-Mg	33.93 ± 2.34 ab	8.35 ± 0.35 ns	4.70 ± 0.14 c	10.20 ± 1.13 ab	35.40 ± 5.12 a	
Peel						
Control	34.26 ± 3.29 ns	-0.55 ± 0.21 a	19.31 ± 4.18 ns	19.40 ± 4.27 ns	94.52 ± 2.73 ns	
-P	31.46 ± 0.91 ns	-1.63 ± 0.04 ab	16.97 ± 2.22 ns	17.03 ± 2.17 ns	94.13 ± 2.82 ns	
-K	33.91 ± 2.97 ns	-1.96 ± 0.33 b	20.35 ± 3.31 ns	20.47 ± 3.34 ns	95.63 ± 0.34 ns	
-Ca	32.06 ± 1.66 ns	-0.79 ± 0.19 ab	13.32 ± 3.14 ns	13.46 ± 3.30 ns	95.19 ± 4.25 ns	
-Mg	35.29 ± 3.53 ns	-1.6 ± 0.70 ab	23.20 ± 5.08 ns	20.51 ± 2.80 ns	91.27 ± 3.09 ns	

Values are the mean of treatments ($n=3$) \pm standard deviation. Different letters in each column means that the treatments were statistically different (Tukey, $p < 0.05$).

On the contrary, the chromatic characteristics of the peel indicate that only significant differences were found in a^* , where only the -K treatment was less than the other treatments (-1.96 ± 0.33), among which no differences were found for this characteristic (Table 2).

The previous results are similar to those reported by Nava *et al.* (2008), who found that, by increasing the doses of K, the value of a^* also increased, as well as a reduction of b^* in apple fruits.

Currently, the effects of macronutrient deficiency on the color parameters of fig fruits are unknown. The main compounds that provide the color to the fruits are carotenoids (orange-yellow colors), anthocyanins

(purple-reddish colors), lycopene (red color), and chlorophylls (green color) (Lancaster *et al.*, 1997). In a study carried out on plants of *Medicago sativa*, an increase in the levels of shikimic acid was found (Rakhmankulova *et al.*, 2003), which is a precursor of some compounds, such as anthocyanins and flavonoids; therefore, by increasing the concentration of these compounds in the fruit, the color parameters change. The differences in color parameters between nutritional deficiency treatments can be explained due to the above.

Botanically, the fig is not a true fruit, but a set of achenes grouped inside a receptacle (peel), therefore, this set of fruits is considered infructescence (Syconium), while the shell corresponds to a modified part of the stem, where the true fruits are found in the pulp (Crisosto *et al.*, 2011). Due to the above, it is explained that the chromatic parameters were affected in the fig pulp, without finding differences in the peel, where only a significant difference was found for a*, which can be explained due to the alteration of the chlorophylls in fruit peels.

Concentration of minerals in fruit

Concentration of elements in fruits was significantly affected ($p < 0.05$) for all the treatments with macronutrient deficiency (Table 3). The concentration of N in the fruits was lower in the -P, -Mg, -K, and -Ca treatments, with reductions compared to the control of 51.4%, 51.4%, 45%, and 32.6%, respectively. The -P, -Ca, and -Mg treatments did not affect the concentration of other elements, however, the -K treatment caused a higher concentration of Mg in the fruit (Table 3).

The increase in the concentration of Mg in fruits with K deficiency is explained by the interaction of the K^+/Mg^{++} ions in the plant, explained by Farhat *et al.* (2016) and Guo *et al.* (2016). This effect has been reported in different plants, such as *Cymbopogon citratus* (Capato *et al.*, 2019) and *Saccharum officinarum* (Rhodes *et al.*, 2018).

Table 3. Macroelement concentrations of fig tree fruits with macronutrient deficiencies

Treatment	Macroelements (% of dry weight)				
	N	P	K	Ca	Mg
Control	2.82 ± 0.29 a	0.17 ± 0.02 ab	0.79 ± 0.08 a	0.23 ± 0.01 ab	0.07 ± 0.01 b
-P	1.37 ± 0.15 b	0.15 ± 0.01 b	0.74 ± 0.12 a	0.28 ± 0.03 ab	0.07 ± 0.01 ab
-K	1.55 ± 0.31 b	0.23 ± 0.02 a	0.38 ± 0.05 b	0.29 ± 0.01 a	0.10 ± 0.01 a
-Ca	1.90 ± 0.42 b	0.22 ± 0.01 a	0.84 ± 0.07 a	0.21 ± 0.02 b	0.09 ± 0.02 ab
-Mg	1.37 ± 0.12 b	0.20 ± 0.02 ab	0.84 ± 0.05 a	0.27 ± 0.03 ab	0.07 ± 0.01 b

Values are the mean of treatments ($n=3$) ± standard deviation. Different letters in each column means that the treatments were statistically different (Tukey, $p < 0.05$).

Regarding the concentration of microelements in fruits, significant differences were found between the treatments for the concentration of Fe, Mn, Zn, and Cu. In the case of the concentration of Fe in the fruits, higher levels were found in the -K treatment (77.69 ± 24.0 ppm) compared to the control (36.82 ± 2.7 ppm), which represents an increase of 111% in the concentration of this element. Fe also increased in the fruits of the -Ca treatment by 50.9% (55.56 ± 10.5 ppm). The same trend was found in the Mn concentrations for the -K and -Ca treatments, which increased by 248.37 (12.89 ± 3.0 ppm) and 201.62% (11.16 ± 2.1 ppm) with respect to the control (3.70 ± 0.3 ppm). Finally, the Cu concentration increased 81.1% in the -P treatment (3.91 ± 0.1 ppm), as well as 72.2% in the -K treatment (3.72 ± 0.3 ppm), the above with respect to the control (2.16 ± 0.3 ppm) (Table 4).

Table 4. Microelement concentrations of fig tree fruits with macronutrient deficiencies

Treatment	Microelements (ppm)				
	Fe	B	Mn	Zn	Cu
Control	36.82 ± 2.7 b	19.16 ± 2.6 ns	3.70 ± 0.3 b	8.38 ± 2.1 ab	2.16 ± 0.3 c
-P	39.54 ± 5.1 b	20.21 ± 2.5 ns	3.38 ± 0.4 b	10.99 ± 2.0 ab	3.91 ± 0.1 a
-K	77.69 ± 24.0 a	22.56 ± 3.3 ns	12.89 ± 3.0 a	11.68 ± 2.7 ab	3.72 ± 0.3 ab
-Ca	55.56 ± 10.5 ab	19.38 ± 3.0 ns	11.16 ± 2.1 a	14.88 ± 2.1 a	2.58 ± 0.1 c
-Mg	44.38 ± 4.4 b	21.85 ± 3.0 ns	4.02 ± 0.4 b	7.72 ± 3.3 b	2.93 ± 0.4 bc

Values are the mean of treatments ($n=3$) ± standard deviation. Different letters in each column means that the treatments were statistically different (Tukey, $p<0.05$).

The aforementioned effects are explained because the plant tends to balance ionic charges inside the cells, so that, as there is a low concentration of K^+ and Ca^{++} ions, a greater accumulation of Fe^{++} , Mn^{++} ions occurs, Zn^{++} and Cu^{++} , all of them cations, as well as K^+ and Ca^{++} (Mengel *et al.*, 2001). Results found in the present investigation coincide with those reported by Garza-Alonso *et al.* (2019), where similarities were observed in the behavior of minerals in the tissues of the fig tree in the absence of macronutrients.

Total phenolic compounds

Concentration of total phenolic compounds, flavonoids, and condensed tannins in the fig pulp resulted with a significant statistical difference ($p<0.05$) between treatments with macronutrient deficiency (Table 5). The lowest concentration of soluble phenols was obtained in the -K treatment (457.61 ± 50.70 mgGAE kg^{-1}), which represented a decrease of 24.4% compared to the control (605.75 ± 61.99 mgGAE kg^{-1}). This effect was also reported by Gaaliche *et al.* (2019), who found a lower concentration of phenols in fig fruits without the application of K, with reductions of 14.3% compared to plants fertilized with this element. On the other hand, Galieni *et al.* (2015) found no difference in the concentration of total soluble phenols between *Lactuca sativa* plants with P deficiency and the control treatment. The same effect was reported by Davarpanah *et al.* (2017) in pomegranate fruits (*Punica granatum*), where no significant difference was found between plants with and without application of Ca.

On the other hand, the bound phenolic compounds increased in the -Ca, -P, and -K treatments by 66.2% (142.43 ± 1.11 mg GAE kg^{-1}), 43.5% (122.97 ± 17.04 mg GAE kg^{-1}), and 30.1% (111.41 ± 16.51 mg GAE kg^{-1}), respectively, compared to the control (85.68 ± 6.80 mg GAE kg^{-1}). This can be explained due to the location of phenolic compounds bound to the cell wall (Acosta *et al.*, 2013), which increased due to the absence of macronutrients, possibly as a protection mechanism for cells. Galieni *et al.* (2015) also reported the increase in the concentration of bound phenolic compounds in plants subjected to P deficiency, where these compounds increased by 20% in lettuce plants (*Lactuca sativa*).

Regarding the concentration of soluble phenolic compounds in the peel of fig fruits, a similar trend was observed as above, where the soluble phenolic compounds in the peel decreased in the -Mg, -K, and -P treatments by 27.4% (278.35 ± 22.83 mg GAE kg^{-1}), 26.6% (282.26 ± 27.89 mg GAE kg^{-1}), and 23.9% (291.86 ± 26.77 mg GAE kg^{-1}), respectively, compared to the control (383.30 ± 16.42 mg GAE kg^{-1}). For fruit peel, bound phenolic compounds decreased in the -Mg and -K treatments by 41.6% (40.78 ± 9.17 mg GAE kg^{-1}) and 21.5% (54.83 ± 1.72 mg GAE kg^{-1}), however, an increase of 66.6% was found in the -Ca treatment (116.43 ± 8.59 mg GAE kg^{-1}), all of the above with respect to the control (69.86 ± 15.00 mg GAE kg^{-1}) (Table 5).

The concentration of soluble flavonoids in pulp was lower in the -K, -Ca, and -Mg treatments, where Ca deficiency negatively affected 78.69%, followed by K with 48.94%, and Mg 33.29%, the above compared with the control. Trejo *et al.* (2019) reported the same effect, where no difference in flavonoid content was found between *Brassica juncea* plants with and without P deficiency. In the research by Gaaliche *et al.* (2019) a 48% reduction in the concentration of total flavonoids was reported when K was not applied to fig plants, as happened in this work. The concentration of bound flavonoids was negatively affected in all the treatments with absence of macronutrients (-P, -K, -Ca, and -Mg), where the lowest concentration was found in the -K

treatment (31.39 ± 2.94 mg CatE kg^{-1}), followed by treatments -Ca (34.22 ± 3.02 mg CatE kg^{-1}), -Mg (36.99 ± 4.91 mg CatE kg^{-1}), and -P (38.33 ± 4.91 mg CatE kg^{-1}). In the case of fruit peel, soluble and bound flavonoids were not detected (Table 5).

Table 5. Phenolic compounds, flavonoids, and condensed tannins of fig tree fruits with macronutrient deficiencies

Treatment	Phenolic compounds (mgGAE kg^{-1})		Flavonoids (mgCatE kg^{-1})		Condensed tannins (mgCatE kg^{-1})	
	Soluble	Bound	Soluble	Bound	Soluble	Bound
Pulp						
Control	605.75 ± 61.99 a	85.68 ± 6.80 b	238.28 ± 29.40 a	51.99 ± 2.89 a	762.58 ± 26.07 c	nd
-P	613.88 ± 30.93 a	122.97 ± 17.04 ab	184.12 ± 11.82 ab	38.33 ± 4.91 b	986.09 ± 7.66 a	nd
-K	457.61 ± 50.70 b	111.41 ± 16.51 ab	121.65 ± 5.87 c	31.39 ± 2.94 b	788.79 ± 10.97 c	nd
-Ca	521.02 ± 27.77 ab	142.43 ± 1.11 a	50.76 ± 22.02 d	34.22 ± 3.02 b	937.56 ± 24.28 ab	nd
-Mg	511.06 ± 54.84 ab	92.08 ± 11.57 b	158.94 ± 8.36 bc	36.99 ± 4.91 b	906.14 ± 21.97 b	nd
Peel						
Control	383.30 ± 16.42 a	69.86 ± 15.00 b	nd	nd	458.15 ± 5.98 bc	nd
-P	291.86 ± 26.77 b	60.46 ± 10.33 bc	nd	nd	422.52 ± 26.08 c	nd
-K	282.26 ± 27.89 b	54.83 ± 1.72 bc	nd	nd	557.66 ± 34.91 a	nd
-Ca	385.09 ± 2.83 a	116.43 ± 8.59 a	nd	nd	539.84 ± 24.05 ab	nd
-Mg	278.35 ± 22.83 b	40.78 ± 9.17 c	nd	nd	455.05 ± 11.59 bc	nd

Values are the mean of treatments ($n=3$) \pm standard deviation. Different letters in each column means that the treatments were statistically different (Tukey, $p<0.05$). nd: not detected.

The condensed tannins were increased in the -P, -Ca, and -Mg treatments with respect to the control. The increase in condensed tannins in the -P, -Ca, and -Mg treatments were 29.3%, 22.9% and 18.8%, respectively. This is explained because the Shikimic Acid pathway can end in the synthesis of anthocyanins or condensed tannins (He *et al.*, 2015). As there is a deficiency of P, plants tend to accumulate anthocyanins in the tissues (Chandra and Karmoker, 2011). In the case of figs with light-colored peels (ripening green or yellow), the content of anthocyanins has not been detected in various studies (Harzallah *et al.*, 2016; Vallejo *et al.*, 2011); on the contrary, this study found an increase in condensed tannins in fruits of the -P treatment, which can be explained by what was previously described. Bound condensed tannins were not detected in pulp and peel of fruits (Table 5).

Antioxidant capacity

The antioxidant capacity of fig fruits was different between treatments ($p<0.05$) for the DPPH, ABTS and FRAP assays (Table 6). In all the evaluations, the soluble portion was the one with the highest levels of antioxidant capacity. For DPPH, the -Ca and -P treatments reported the highest levels of antioxidant capacity, with increases of 12.8% (3265.1 ± 30.4 $\mu\text{molTE kg}^{-1}$) and 9.0% (3127.6 ± 11.0 $\mu\text{molTE kg}^{-1}$), with respect to the control (2844.8 ± 35.4 $\mu\text{molTE kg}^{-1}$). In the case of the bound portion, the fruits with Ca deficiency had a higher antioxidant capacity, with an increase of 40.83% (788.4 ± 14.3) compared to the control (559.8 ± 30.7) (Table 6).

Table 6. Antioxidant capacity (DPPH, ABTS, and FRAP) of fig tree fruits with macronutrient deficiencies

Treatment	DPPH ($\mu\text{molTE kg}^{-1}$)		ABTS ($\mu\text{molTE kg}^{-1}$)		FRAP ($\mu\text{molTE kg}^{-1}$)	
	Soluble	Bound	Soluble	Bound	Soluble	Bound
Pulp						
Control	2844.8 \pm 35.4 c	559.8 \pm 30.7 b	4337.1 \pm 41.4 b	646.9 \pm 23.6 c	983.3 \pm 55.3 c	120.4 \pm 42.0 c
-P	3127.6 \pm 11.0 b	599.2 \pm 13.6 b	4501.8 \pm 36.1 a	740.8 \pm 21.1 b	697.6 \pm 65.7 d	693.4 \pm 33.5 ab
-K	2528.1 \pm 35.1 d	585.3 \pm 21.6 b	3202.7 \pm 20.8 d	886.2 \pm 18.9 a	1226.1 \pm 65.8 b	669.7 \pm 43.8 ab
-Ca	3265.1 \pm 30.4 a	788.4 \pm 14.3 a	3904.1 \pm 17.0 c	841.4 \pm 17.1 a	686.0 \pm 38.8 d	560.2 \pm 56.8 b
-Mg	2732.5 \pm 23.1 c	647.2 \pm 37.5 b	4366.4 \pm 65.0 b	694.7 \pm 20.3 bc	1453.1 \pm 45.0 a	726.5 \pm 17.7 a
Peel						
Control	1396.7 \pm 26.3 b	511.3 \pm 20.7 a	2257.2 \pm 23.5 b	487.8 \pm 61.3 b	2561.9 \pm 47.1 c	196.5 \pm 56.3 c
-P	1371.6 \pm 72.3 b	352.1 \pm 27.3 b	2147.1 \pm 21.4 b	329.9 \pm 42.1 c	3354.6 \pm 61.6 b	226.5 \pm 29.3 c
-K	1002.7 \pm 61.4 c	389.5 \pm 23.6 b	1241.7 \pm 31.2 c	372.9 \pm 20.8 bc	3821.8 \pm 115.1 a	395.2 \pm 20.1 b
-Ca	1977.6 \pm 12.5 a	561.0 \pm 22.2 a	2803.3 \pm 24.1 a	695.8 \pm 24.1 a	2539.7 \pm 36.1 c	429.5 \pm 12.8 ab
-Mg	966.6 \pm 71.6 c	264.6 \pm 32.8 c	2173.8 \pm 54.2 b	259.1 \pm 54.2 c	3251.9 \pm 27.5 b	470.0 \pm 12.9 a

Values are the mean of treatments (n=3) \pm standard deviation. Different letters in each column means that the treatments were statistically different (Tukey, $p < 0.05$).

The ABTS assay showed a similar trend, since the fruits of the -P treatment presented the highest antioxidant capacity ($4501.8 \pm 36.1 \mu\text{molTE kg}^{-1}$), which represented an increase of 3.8% compared to the control ($4337.1 \pm 41.4 \mu\text{molTE kg}^{-1}$), while the lowest values were reported in the -K (3202.7 ± 20.8) and -Ca ($3904.1 \pm 17.0 \mu\text{molTE kg}^{-1}$) treatments compared to the -P treatment. The ABTS antioxidant capacity in the bound compounds had an inverse behavior to the soluble compounds, because it increased in the -K and -Ca treatments by 37 and 30%, respectively, compared to the control (Table 6).

Fruit peel and pulp resulted similar for antioxidant capacity, since the -K treatment indicated the lowest values of antioxidant capacity in the soluble portion ($1241.7 \pm 31.2 \mu\text{molTE kg}^{-1}$), while the -Ca treatment presented the highest antioxidant capacity values for the soluble and bound portion, with increases of 24.2% ($2803.3 \pm 24.1 \mu\text{molTE kg}^{-1}$) and 42.7% ($695.8 \pm 24.1 \mu\text{molTE kg}^{-1}$), respectively, compared to the values obtained in control (2257.2 ± 23.5 and $487.8 \pm 61.3 \mu\text{molTE kg}^{-1}$). In contrast, the lowest values in the bound portion were found in the -Mg treatments ($259.1 \pm 54.2 \mu\text{molTE kg}^{-1}$), -P ($329.9 \pm 42.1 \mu\text{molTE kg}^{-1}$), and -K ($372.9 \pm 20.8 \mu\text{molTE kg}^{-1}$), with respect to the control ($487.8 \pm 61.3 \mu\text{molTE kg}^{-1}$) (Table 6).

The FRAP assay found an increase in the antioxidant capacity of the pulp in the soluble portion of 47.8% for the -Mg treatment ($1453.1 \pm 45.0 \mu\text{molTE kg}^{-1}$) and 24.7% for -K ($1226.1 \pm 65.8 \mu\text{molTE kg}^{-1}$), with respect to the control ($983.3 \pm 55.3 \mu\text{molTE kg}^{-1}$), while the -Ca and -P treatments decreased by 30 and 29%, respectively, compared to the control ($983.3 \pm 55.3 \mu\text{molTE kg}^{-1}$) (Table 6).

In the case of fruit pulp and peel bound compounds, higher antioxidant capacity values were found for all macronutrient deficiency treatments (-P, -K, -Ca, and -Mg). For pulp, the values were reported in the following order: -Mg > -P > -K > -Ca, with increases of 503.4% ($726.5 \pm 17.7 \mu\text{molTE kg}^{-1}$), 475.9% ($693.4 \pm 33.5 \mu\text{molTE kg}^{-1}$), 456.22% ($669.7 \pm 43.8 \mu\text{molTE kg}^{-1}$), and 365% ($560.2 \pm 56.8 \mu\text{molTE kg}^{-1}$), respectively, compared to the control ($120.4 \pm 42.0 \mu\text{molTE kg}^{-1}$). On the other hand, increases in fruit peel were reported in the following order: -Mg > -Ca > -K, with increases of 139.2% ($470.0 \pm 12.9 \mu\text{molTE kg}^{-1}$), 118.6% ($429.5 \pm 12.8 \mu\text{molTE kg}^{-1}$), and 101.1% ($395.2 \pm 20.1 \mu\text{molTE kg}^{-1}$), respectively, compared to the control ($196.5 \pm 56.3 \mu\text{molTE kg}^{-1}$) (Table 6).

In general, the soluble FRAP values were higher in peel, compared to pulp, for all the treatments evaluated. The above coincides with that reported by Guo *et al.* (2003) where a higher antioxidant capacity was found in the peel of different fruits, such as orange, pineapple, and banana. The FRAP antioxidant capacity in the soluble portion of peel increased in the -K, -P, and -Mg treatments by 49.2, 31.0, and 27.0 %, respectively, compared to the control (256.2 ± 4.71).

The reduction in antioxidant capacity using the DPPH assay is explained by the low amount of total phenolic compounds in fruits with absence of K. On the other hand, Kumar *et al.* (2007) reported increases in the free radical H₂O₂ in *Morus alba* fruits subjected to P deficiency; this effect could explain the increase in DPPH antioxidant capacity in the fruits of the -P treatment of the present study. On the other hand, Guo *et al.* (2018) reported an increase of 15% in the antioxidant capacity in plants of *Brassica oleracea* with Ca deficiency. Singh *et al.* (2011) found greater antioxidant capacity in *Dacus carota* plants with Ca deficiency, which increased by 50%, determined by the ORAC method for water-soluble compounds, which can be compared with the DPPH assay used in the present study. A similar trend was found in the case of antioxidant capacity in shell.

The increase in ABTS antioxidant capacity was also reported by Galieni *et al.* (2015) when finding a 25% increase in ABTS antioxidant capacity in *Lactuca sativa* plants without application of P. The same effect was reported by Singh *et al.* (2011) in *Dacus carota* using the ORAC test for fat soluble compounds, where an increase of 17.64% was observed, which can be compared with the ABTS test. Another study by Ahmad *et al.* (2016) reported increases in H₂O₂ levels and increased lipid peroxidation in *Cicer arietinum* plants subjected to Ca deficiency, which could explain the results found in our research. Ca is an important element in plant metabolism, since one of its main functions is as a component of the cell membrane, which favors its integrity (Hawkesford *et al.*, 2012). Due to the above, Ca deficiency promotes oxidative stress in plant cells. This effect was widely described by Nieves *et al.* (2018).

Michalska *et al.* (2016) reported an increase in FRAP in K-deficient *Solanum tuberosum* plants, as in the present study. FRAP antioxidant capacity also decreased in *Brassica oleracea* plants with absence of Ca (Guo *et al.*, 2018). The increase in FRAP antioxidant capacity for the bound portion can be explained by a response of the plant to avoid cell damage by free radicals, increasing the antioxidant capacity of compounds bound to the cell wall.

Conclusions

The absence of macronutrients in fig plants during vegetative and reproductive growth affected performance, reducing the size, weight, and number of fruits, while plants with N deficiencies did not produce fruits. Likewise, macronutrient deficiencies affected the physicochemical quality of the fruits, with reductions in °Brix, pH, and AT. It is important to mention that the total phenolic compounds (soluble + bound) increased in the -P and -Ca treatments, which was reflected in increases in antioxidant capacities in the DPPH, ABTS, and FRAP tests. The -K treatment registered the lowest values of phenolic compounds and, therefore, of antioxidant capacity. Based on the results, it is concluded that each element had a specific effect on fruit yield and quality.

Authors' Contributions

Conceptualization: CAGA and EOS; Data curation: GNM and EOS; Funding acquisition: AGD, Investigation: REVA and ALJ; Methodology: JIGL, AGD and ALJ; Project administration: EOS; Supervision: JIGL, REVA and ALJ; Writing - original draft: CAGA; Writing - review and editing: GNM and EOS. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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